BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

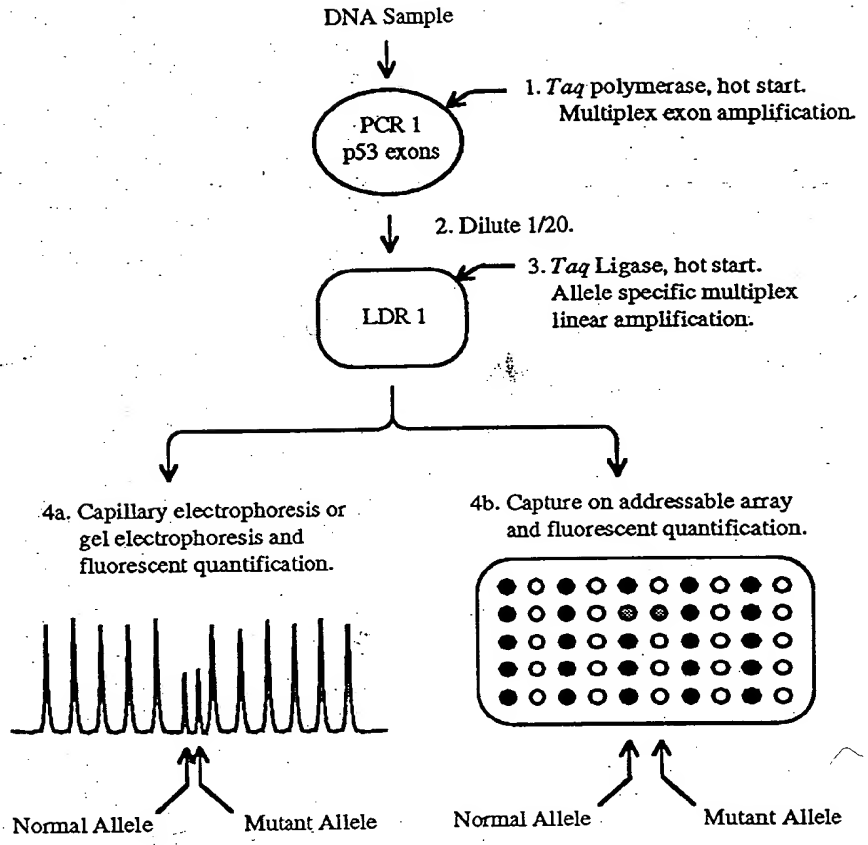

DNA Sample
1. Taq polymerase, hot start.
Multiplex exon amplification.
PCR 1
p53 exons
2. Dilute 1/20.
3. Taq Ligase, hot start.
Allele specific multiplex
linear amplification.
LDR 1
4a. Capillary electrophoresis or
gel electrophoresis and
fluorescent quantification.
4b. Capture on addressable array
and fluorescent quantification.
Normal Allele
Mutant Allele
Normal Allele
Mutant Allele

FIGURE 1

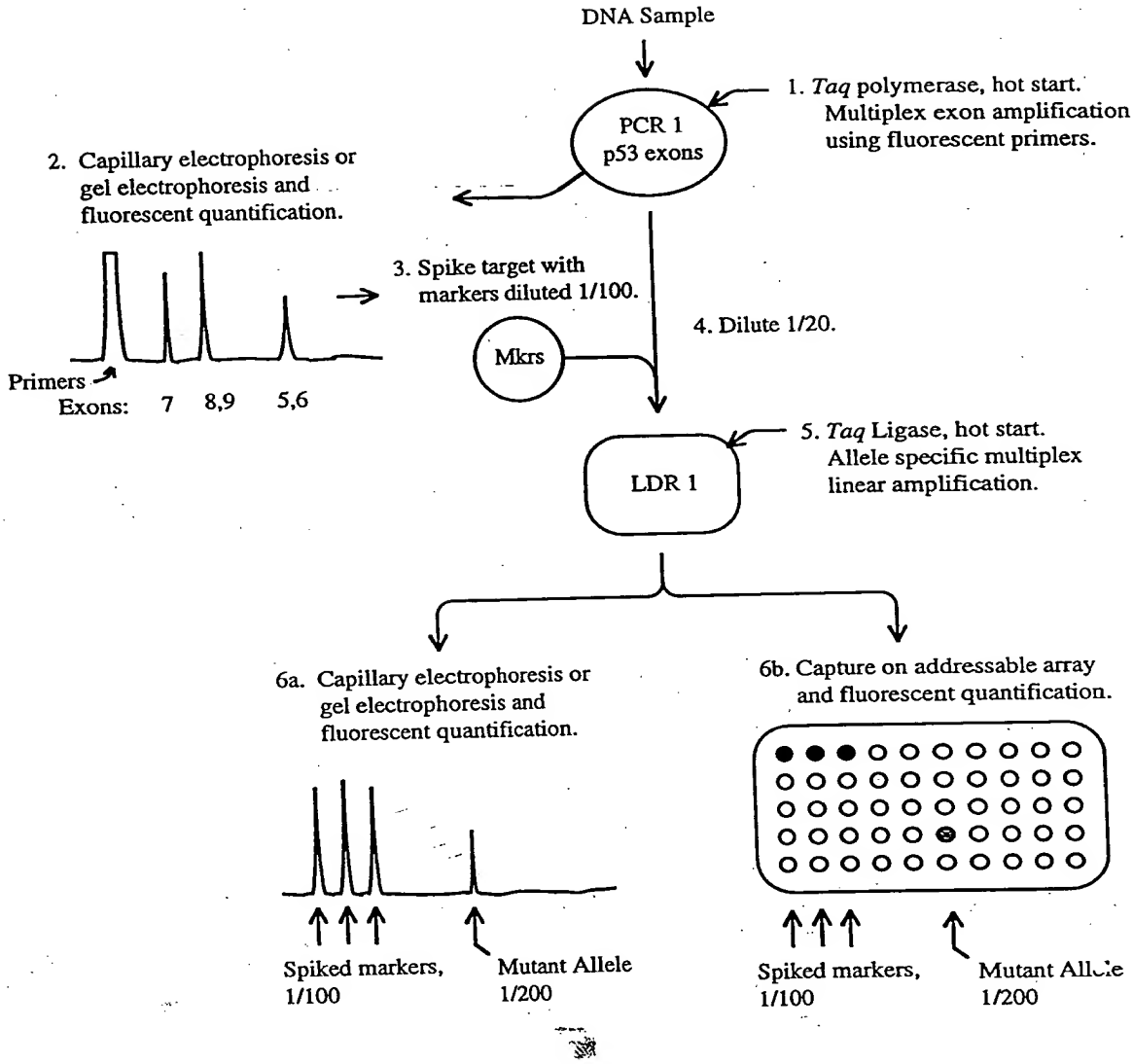

DNA Sample
1. *Taq* polymerase, hot start. Multiplex exon amplification using fluorescent primers.
PCR 1
p53 exons
2. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
3. Spike target with markers diluted 1/100.
Mkrs
4. Dilute 1/20.
Primers
Exons: 7 8,9 5,6
5. *Taq* Ligase, hot start. Allele specific multiplex linear amplification.
LDR 1
6a. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
6b. Capture on addressable array and fluorescent quantification.
Spiked markers, 1/100
Mutant Allele 1/200
Spiked markers, 1/100
Mutant Allele 1/200

FIGURE 2

PCR/ LDR

1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase. ◆

2. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

3. Capture fluorescent products on addressable array and quantify each allele.

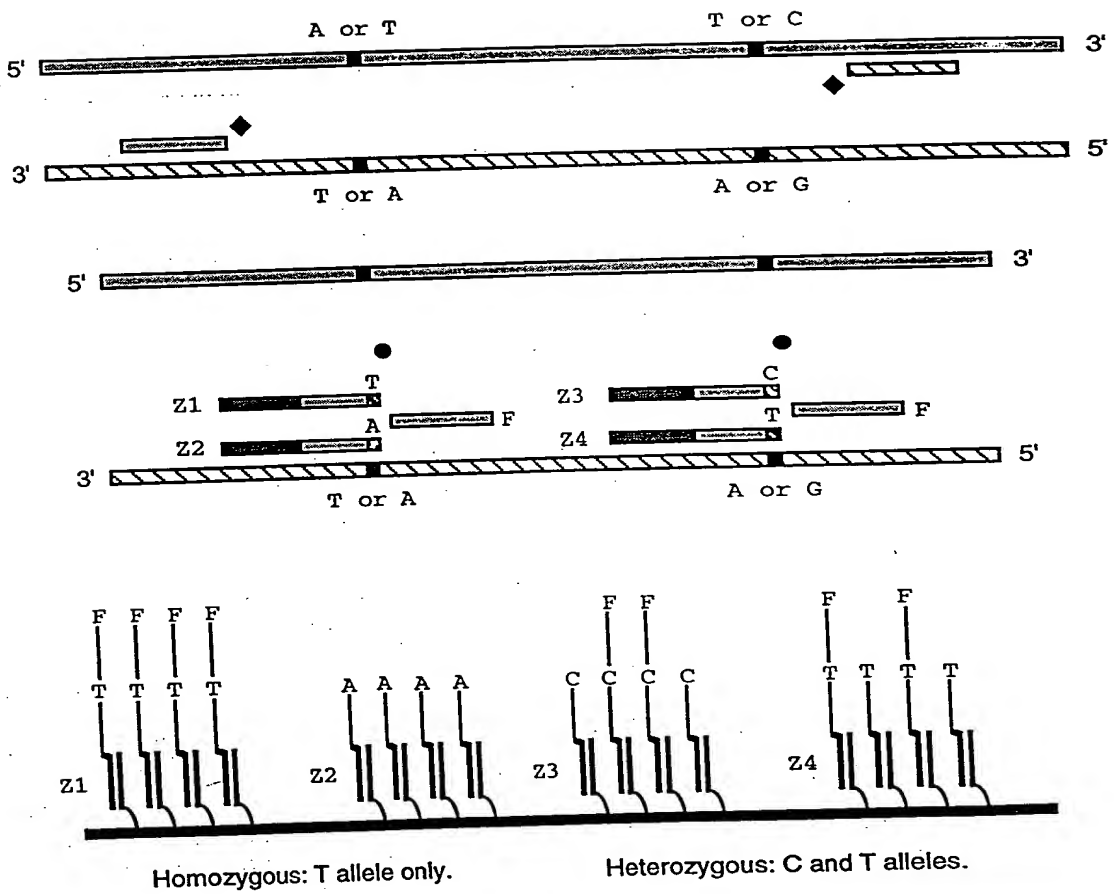
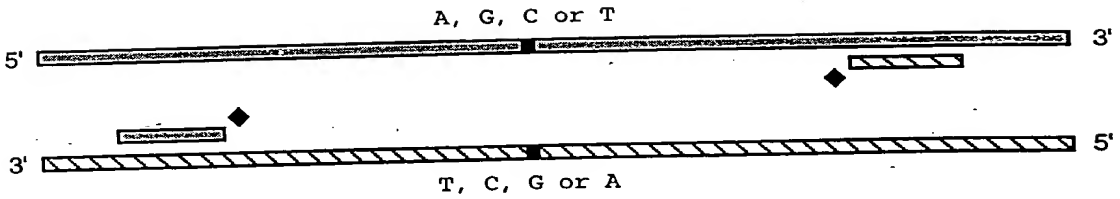


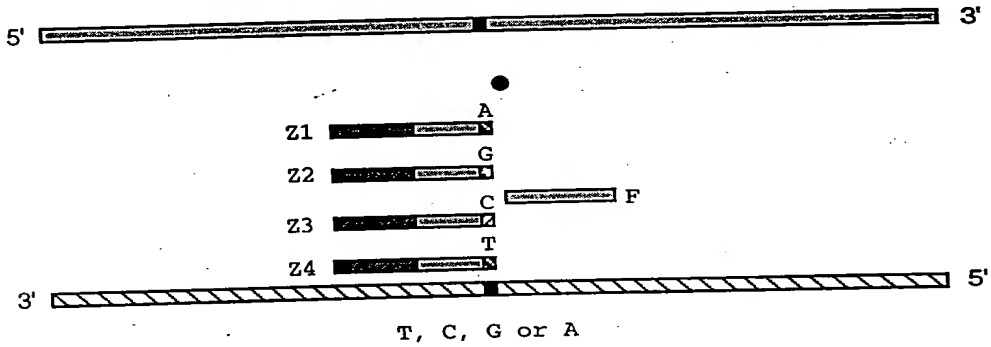
FIGURE 3

PCR/ LDR

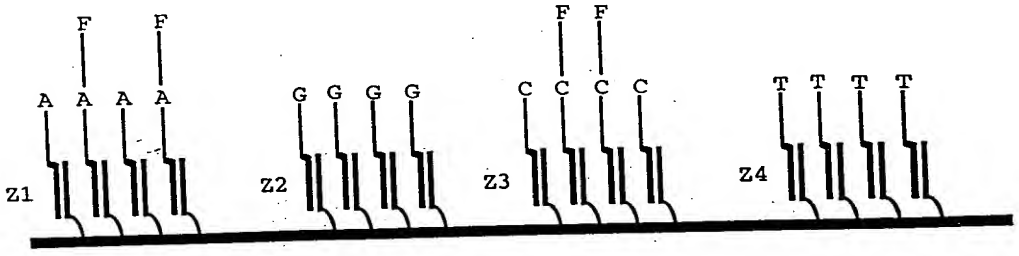
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase. ◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.

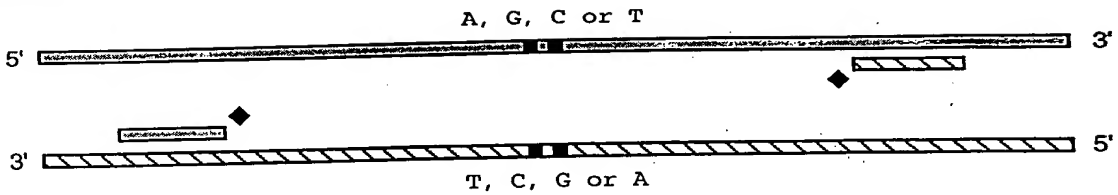


Heterozygous: A and C alleles.

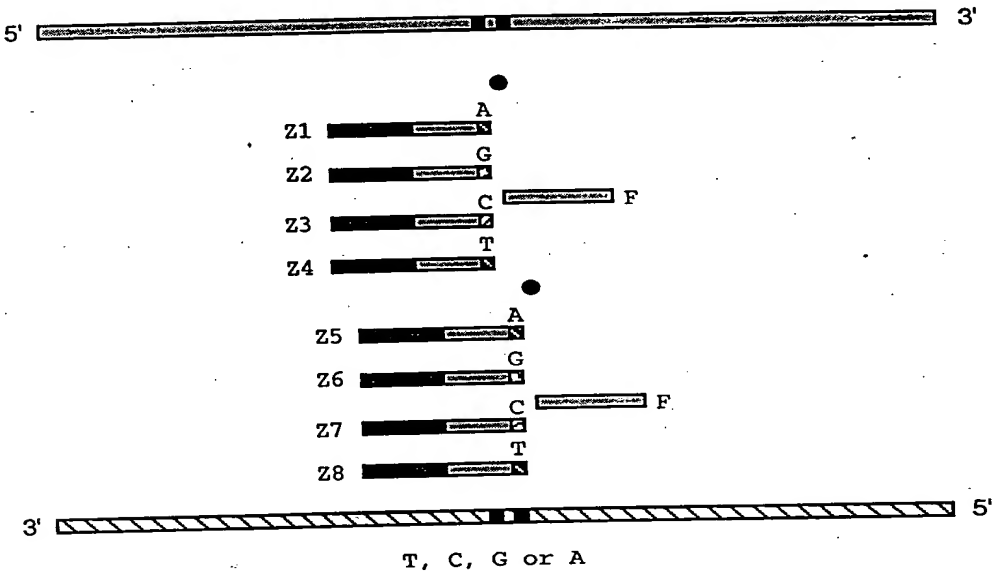
FIGURE 4

PCR/ LDR : Nearby alleles

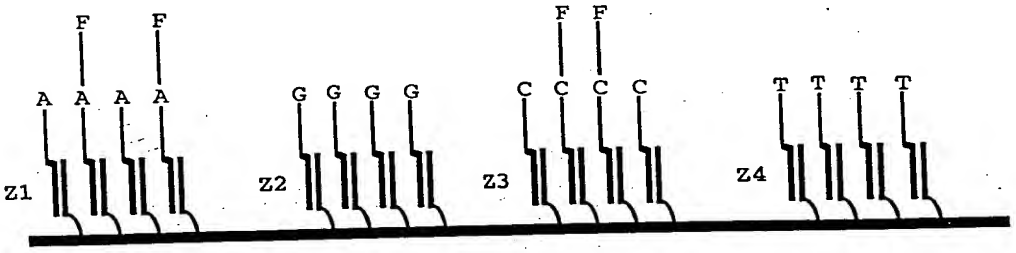
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase. ◆



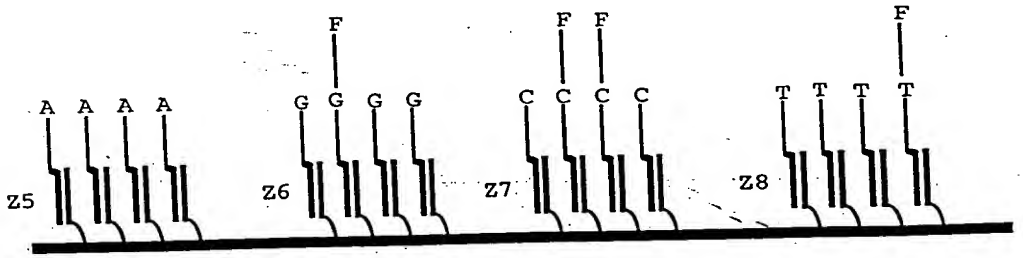
2. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A and C alleles.

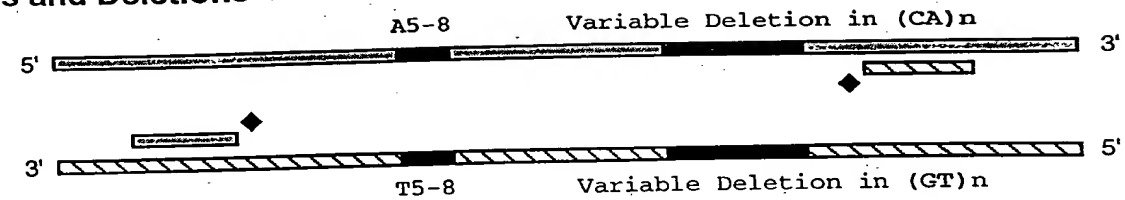


Heterozygous: G,C, and T alleles.

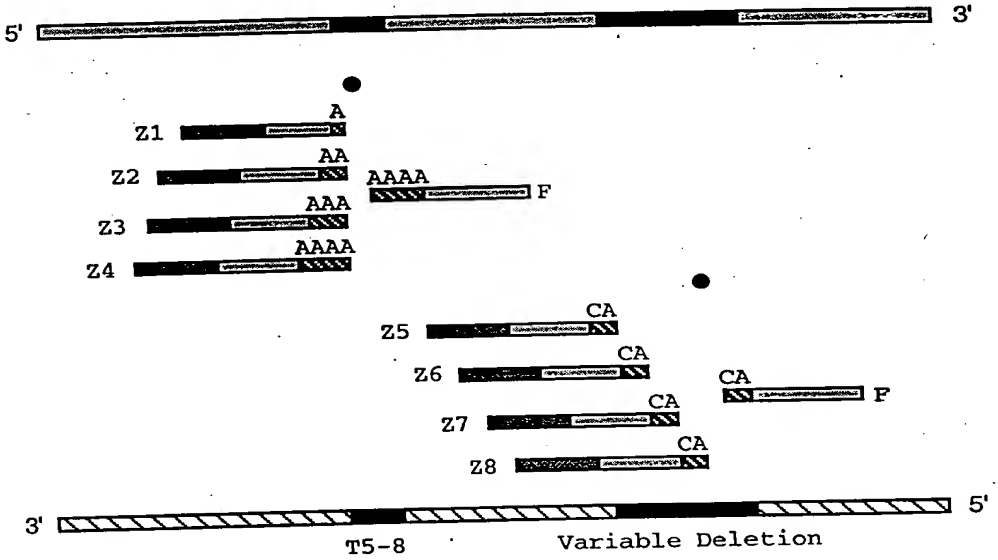
FIGURE 5

PCR/ LDR : Insertions and Deletions

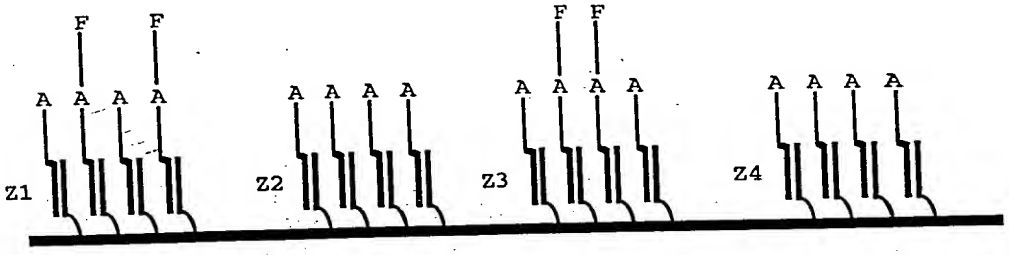
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



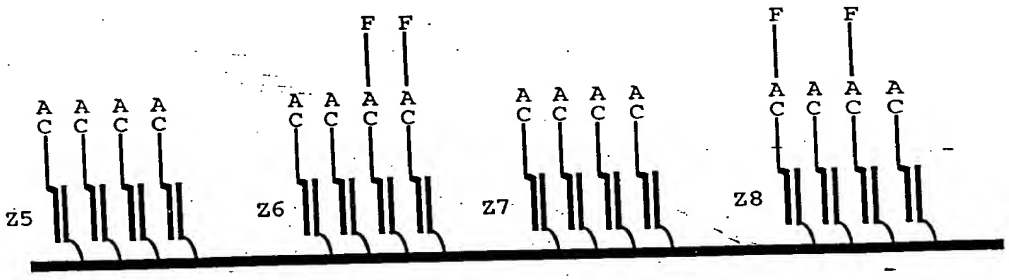
2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A5 and A7 alleles.

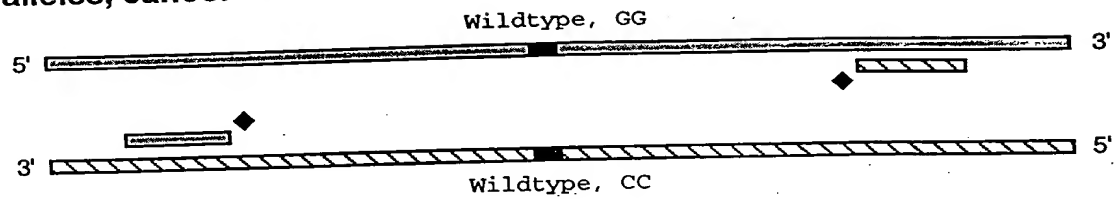


Heterozygous: (CA)5 and (CA)3 alleles.

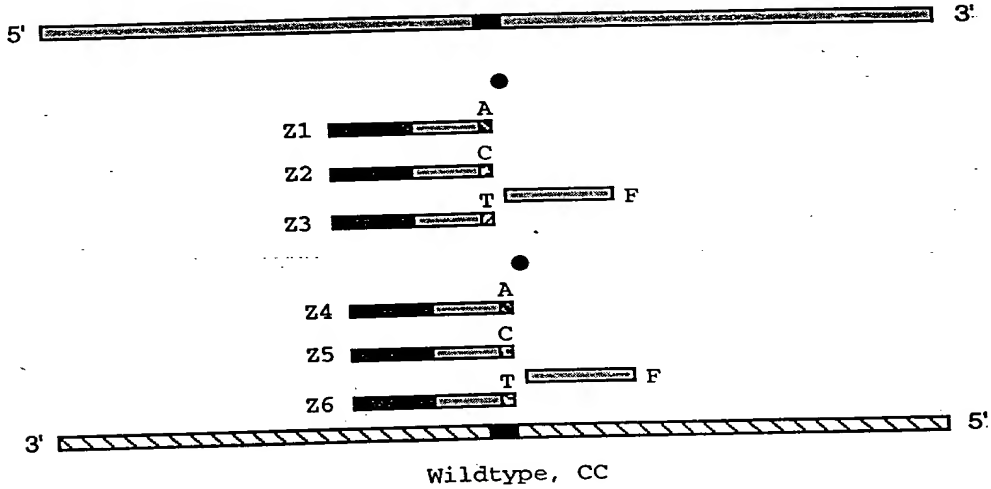
FIGURE 6

PCR/ LDR : Adjacent alleles, cancer detection

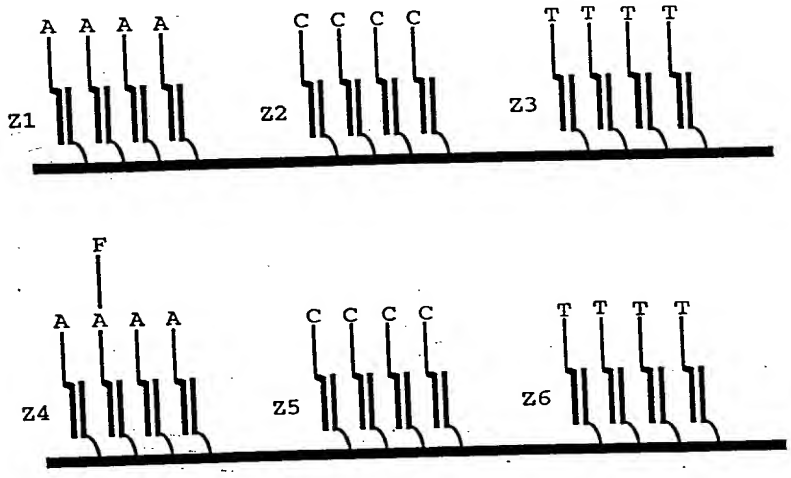
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.

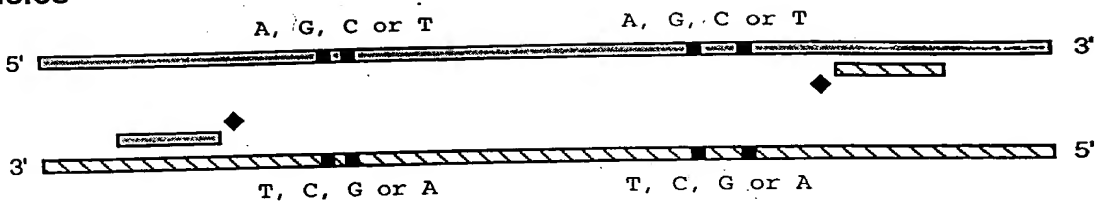


Gly to Asp mutation

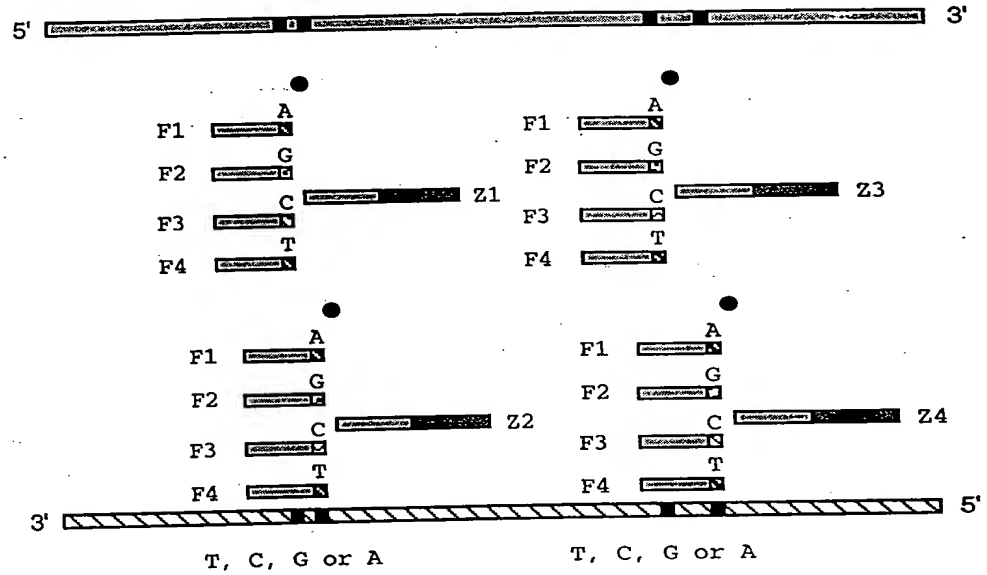
FIGURE 7

PCR/ LDR : Nearby alleles

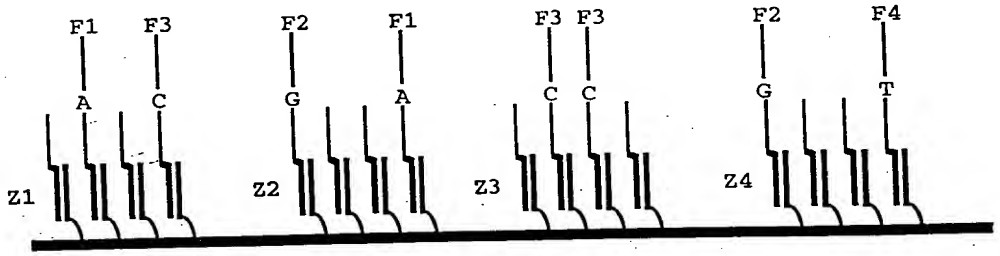
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase. ◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A and C alleles.

Heterozygous: A and G alleles.

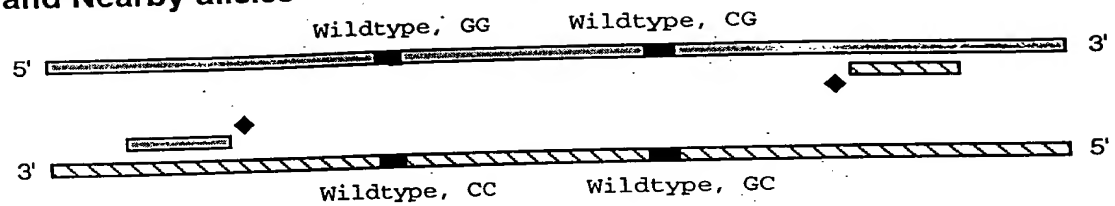
Homozygous: C allele.

Heterozygous: G and T alleles.

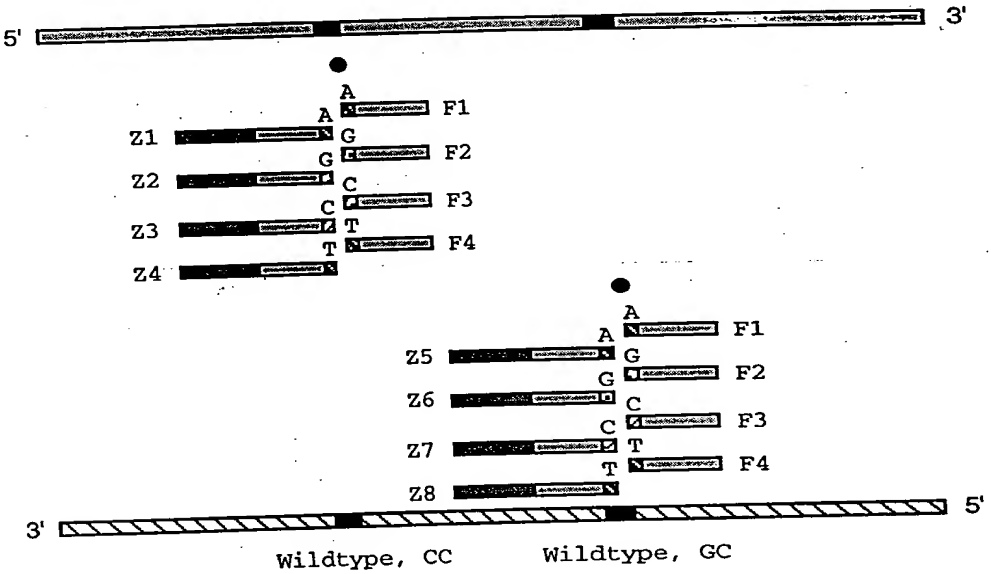
FIGURE 8

PCR/ LDR : Adjacent and Nearby alleles

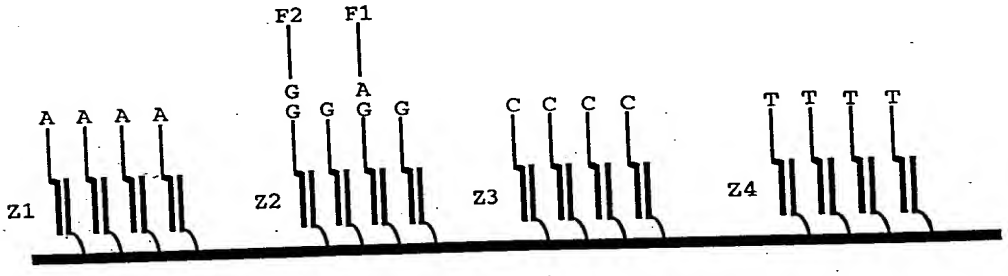
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



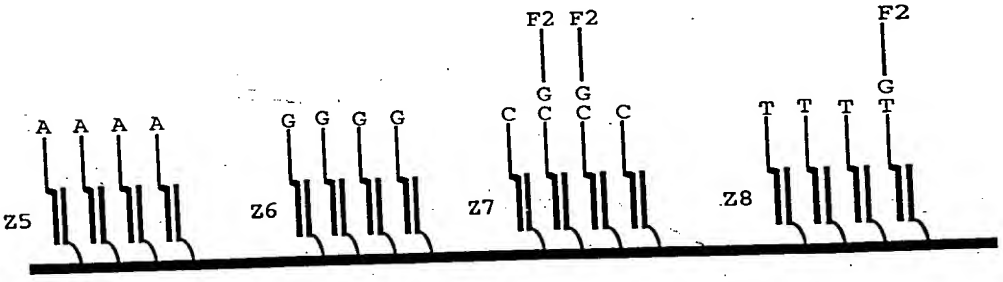
2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: Gly and Glu alleles.



Heterozygous: Arg and Trp alleles.

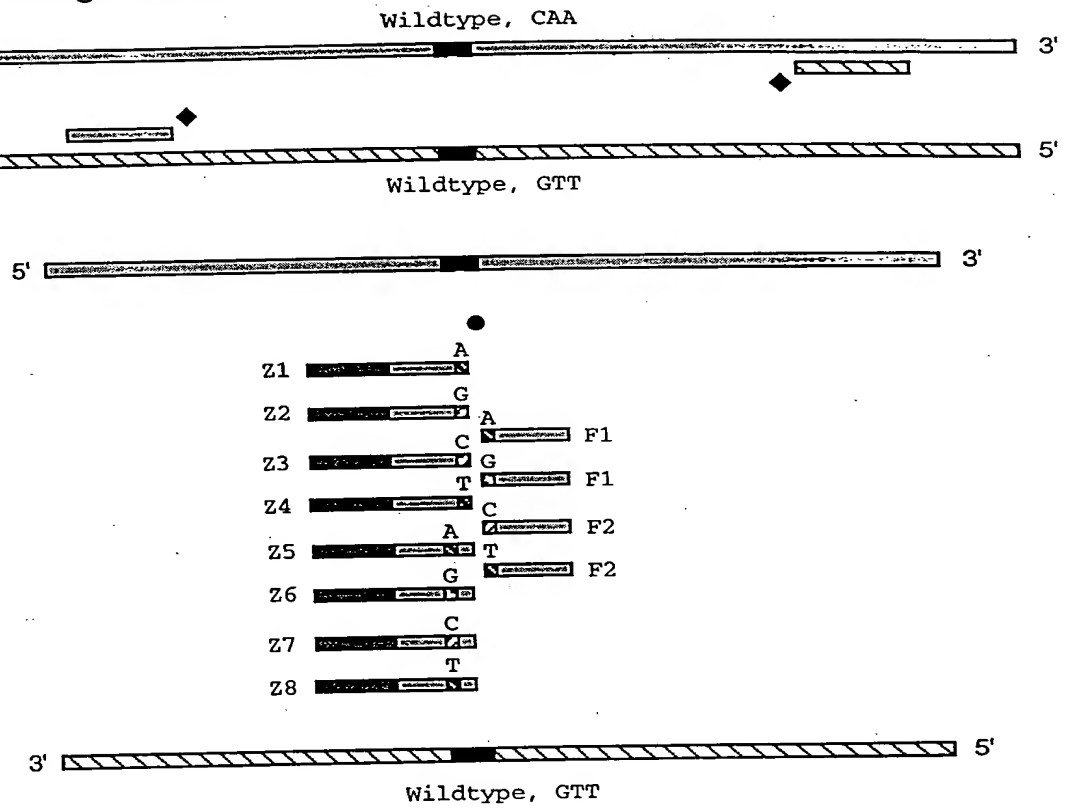
FIGURE 9

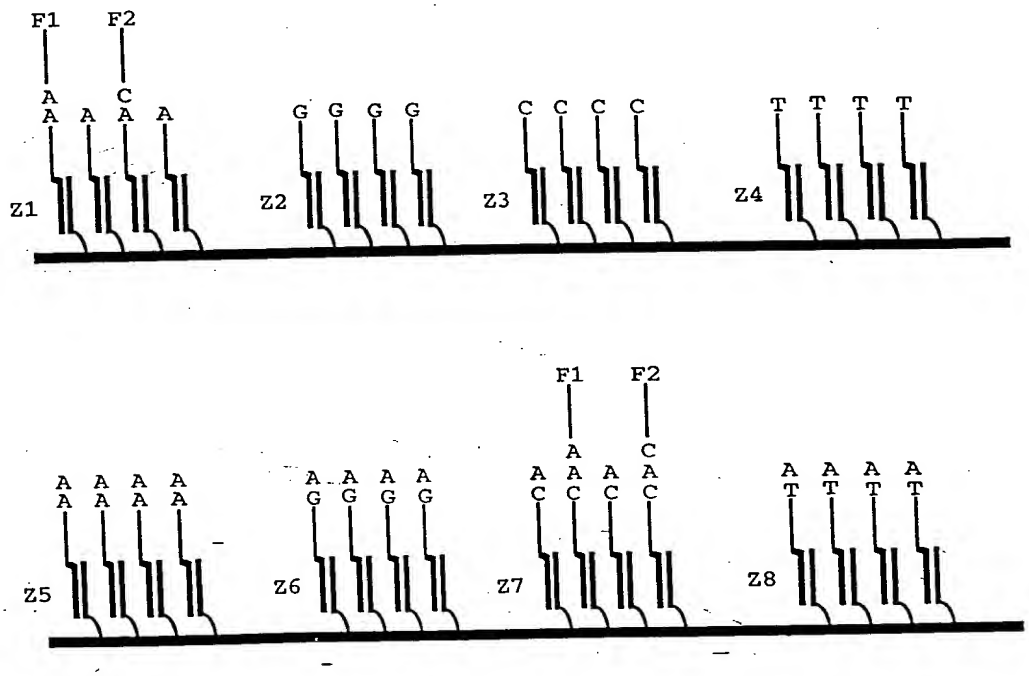
PCR/ LDR : All alleles of a single codon

1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆

2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

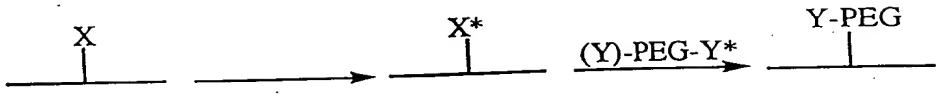
3. Capture fluorescent products on addressable array and quantify each allele.





Heterozygous: Gln and His alleles.

FIGURE 10



X,Y = –OH, –CO_2H, –NH_2

(Y) = W–NH–

X*, Y* = –O(C=O)Z, –O(C=S)Z, –CO_2H, –(C=O)Z, –NH_2, –N=C=O

W = protecting group, e.g. Boc, Fmoc

Z = activating group, e.g. imidazole (Im), *p*-nitrophenol (OPnp), hydroxysuccinimide (OSu), pentafluorophenol (OPfp)

PEG = oligo or poly(ethylene glycol), backbone $(CH_2CH_2O)_n$ n = 6 to 200 (can also be grown by anionic polymerization with )

WSC = water soluble carbodiimide

Functional group transformations/activation (as needed), X → X*, Y → Y*

–OH ⟶ $–O(CH_2)_nCO_2H$ n = 1, 2

–OH ⟶ $–O(C=O)NHCH_2CO_2H$

–OH ⟶ $–O(C=O)CH_2NH_2$

–OH ⟶ –O(C=O)Im

–OH ⟶ $–O(C=S)SCH_2(C=O)NH_2$

$–CO_2H$ ⟶ $–(C=O)NH(CH_2)_nNH_2$ n = 2,6

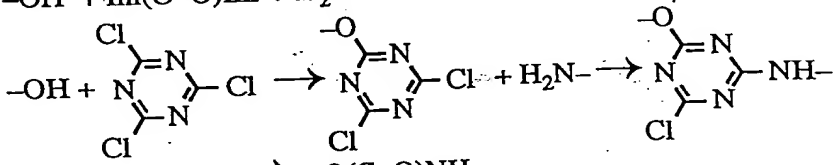
$–CO_2H$ ⟶ –(C=O)Z

$–NH_2$ ⟶ $–NH(C=O)(CH_2)_nCO_2H$ n = 2, 3

Covalent linkage, X* + Y*

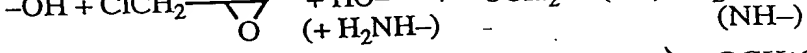
$–CO_2H + H_2N– + WSC + HOSu \longrightarrow –(C=O)NH–$

$–OH + Im(C=O)Im + H_2N– \longrightarrow –O(C=O)NH–$



$–OH + O=C=N– \longrightarrow –O(C=O)NH–$

$–O(C=S)SCH_2(C=O)NH_2 + H_2N– \longrightarrow –O(C=S)NH–$



$–OH \longrightarrow –OCH_2(C=O)H + H_2N– + NaCNBH_3 \longrightarrow –OCH_2CH_2NH–$

FIGURE 11

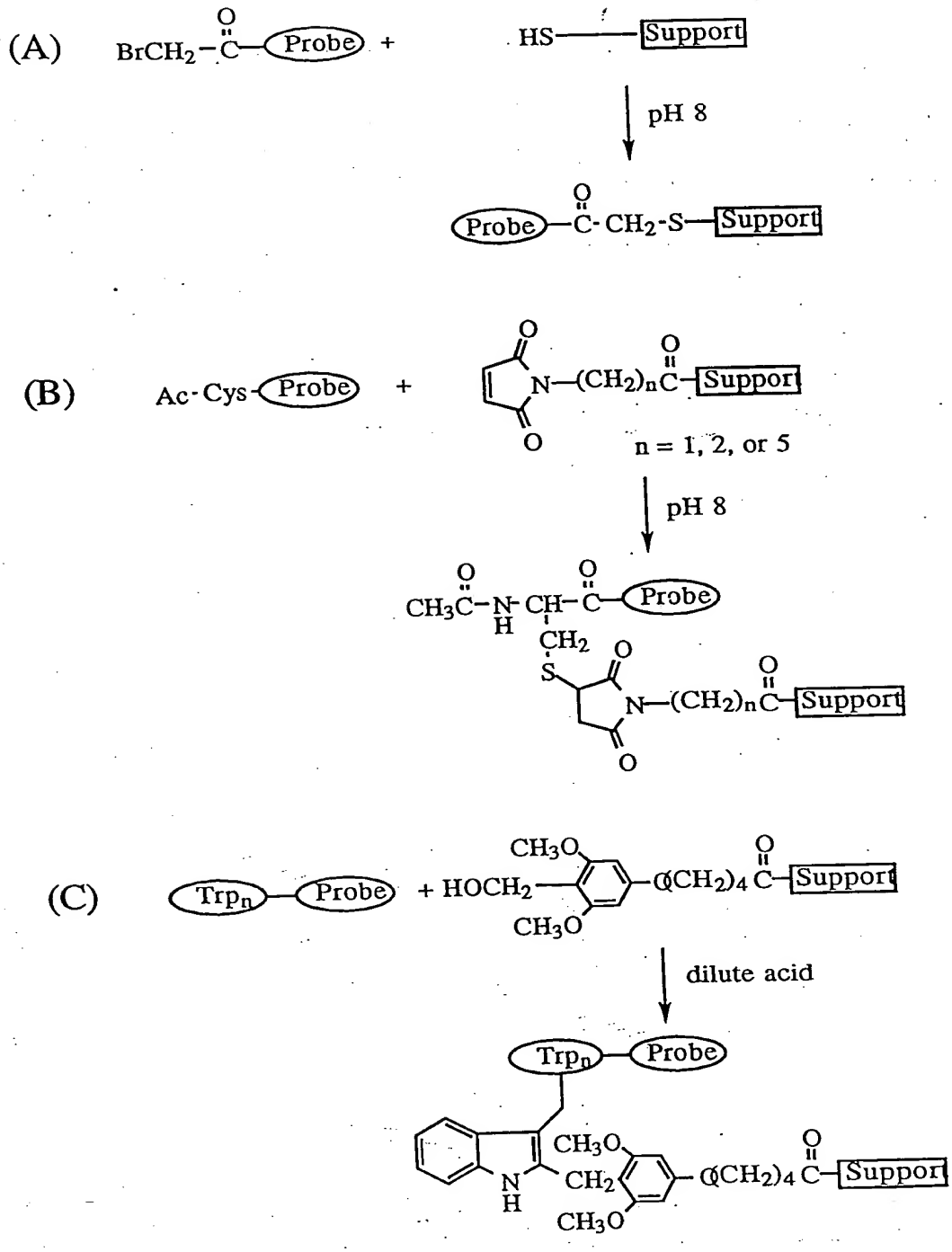
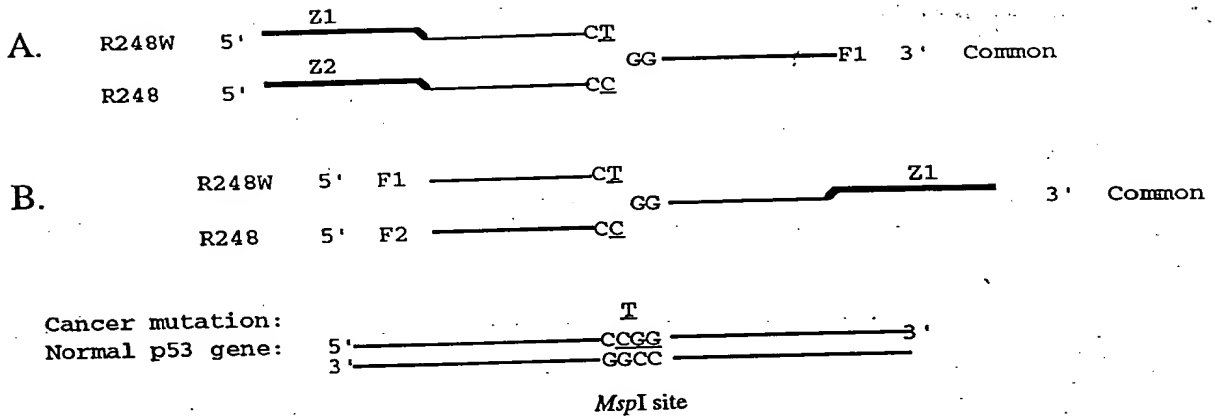

(A)
BrCH2–C(=O)–Probe +
HS–Support
pH 8
Probe–C(=O)–CH2–S–Support
(B)
Ac-Cys-Probe +
n = 1, 2, or 5
pH 8
CH3C(=O)–N(H)–CH(CH2)–C(=O)–Probe
S
N–(CH2)n–C(=O)–Support
(C)
Trpn–Probe + HOCH2
CH3O
CH3O
(CH2)4 C(=O)–Support
dilute acid
Trpn–Probe
CH3O
CH2
CH3O
N
H
(CH2)4 C(=O)–Support

FIGURE 12


A.
R248W
5'
Z1
CT
GG
F1
3'
Common
R248
5'
Z2
CC
B.
R248W
5'
F1
CT
Z1
GG
3'
Common
R248
5'
F2
CC
Cancer mutation:
Normal p53 gene:
T
5'
CCGG
3'
3'
GGCC
MspI site

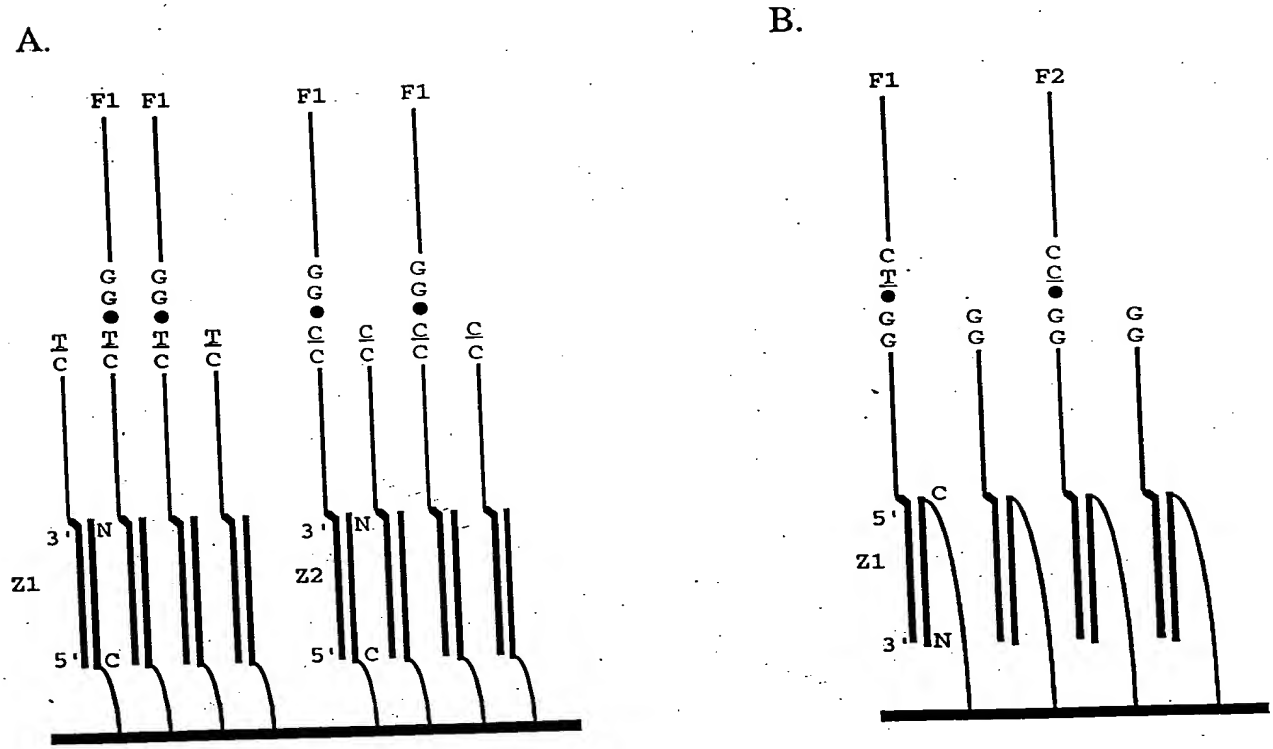
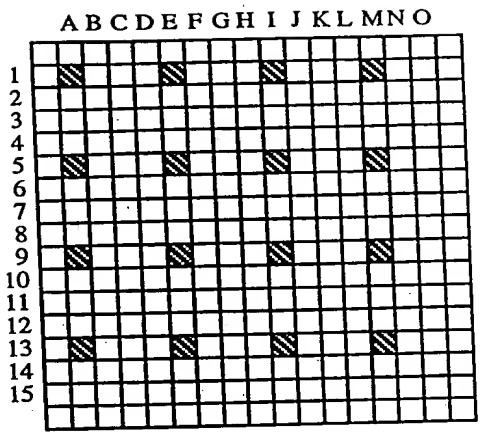
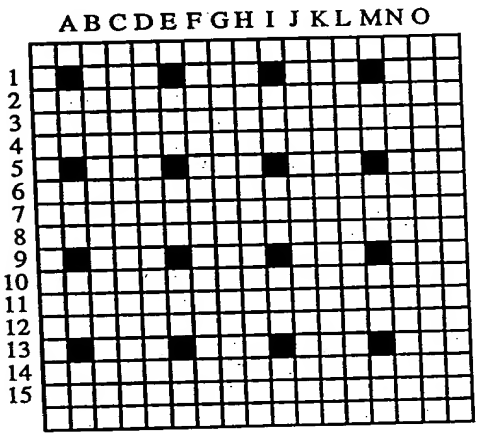

A.
F1
F1
F1
F1
3'
N
Z1
5'
C
Z2
B.
F1
F2
C
5'
Z1
3'
N

FIGURE 13

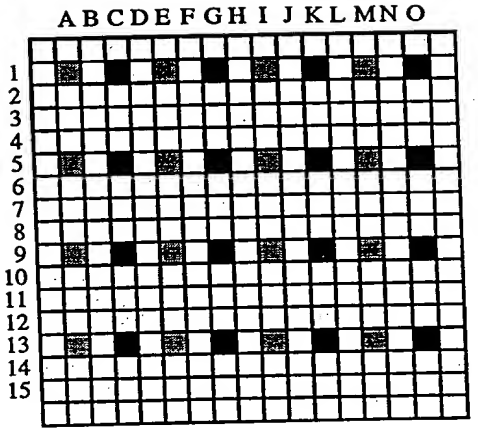
A



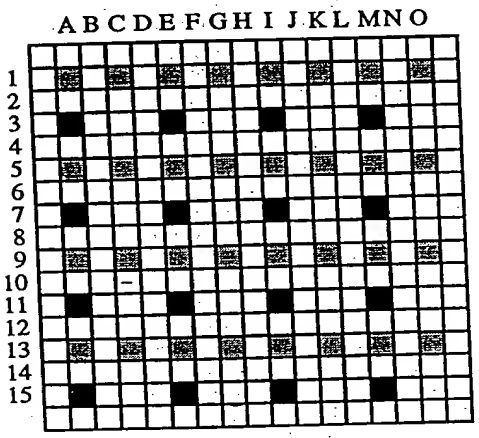
B



C



D



E

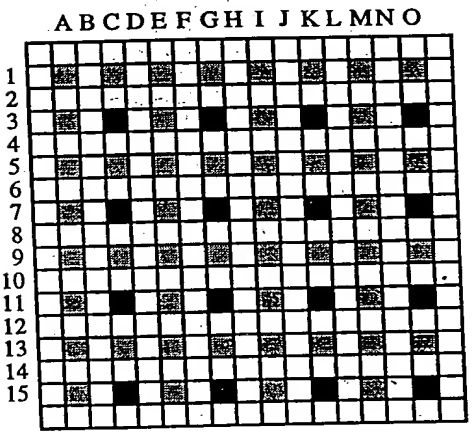
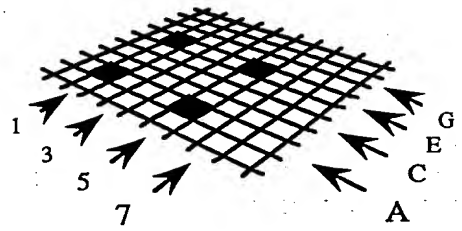
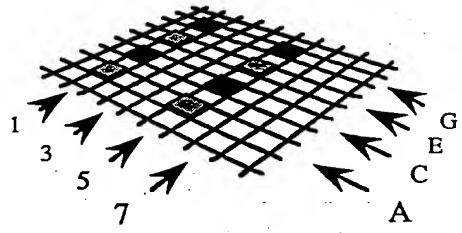


FIGURE 14

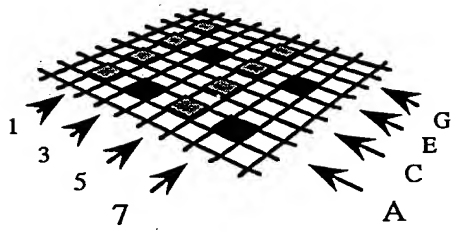
A. 1st addition of unique 24mers.



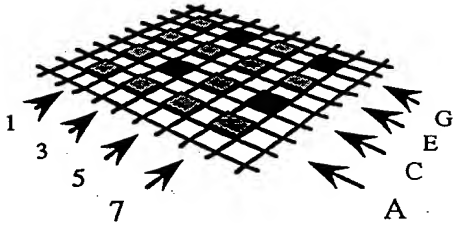
B. 2nd addition of unique 24mers.



C. 3rd addition of unique 24mers.



D. 4th addition of unique 24mers.



E.

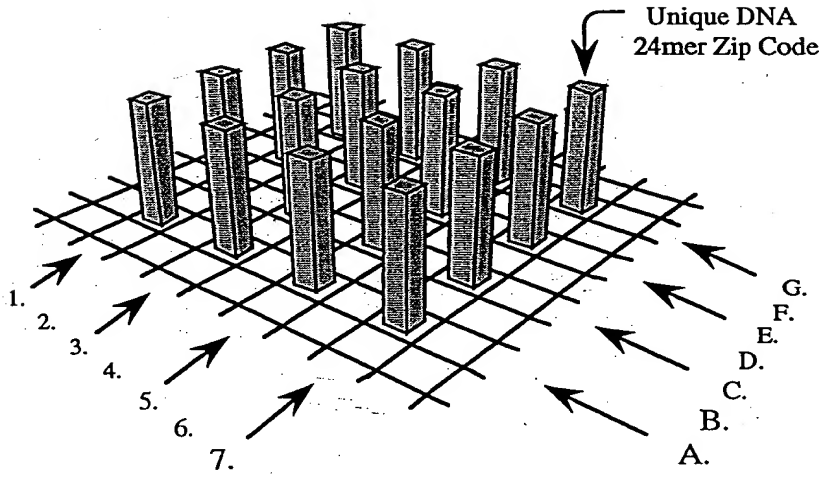


FIGURE 15

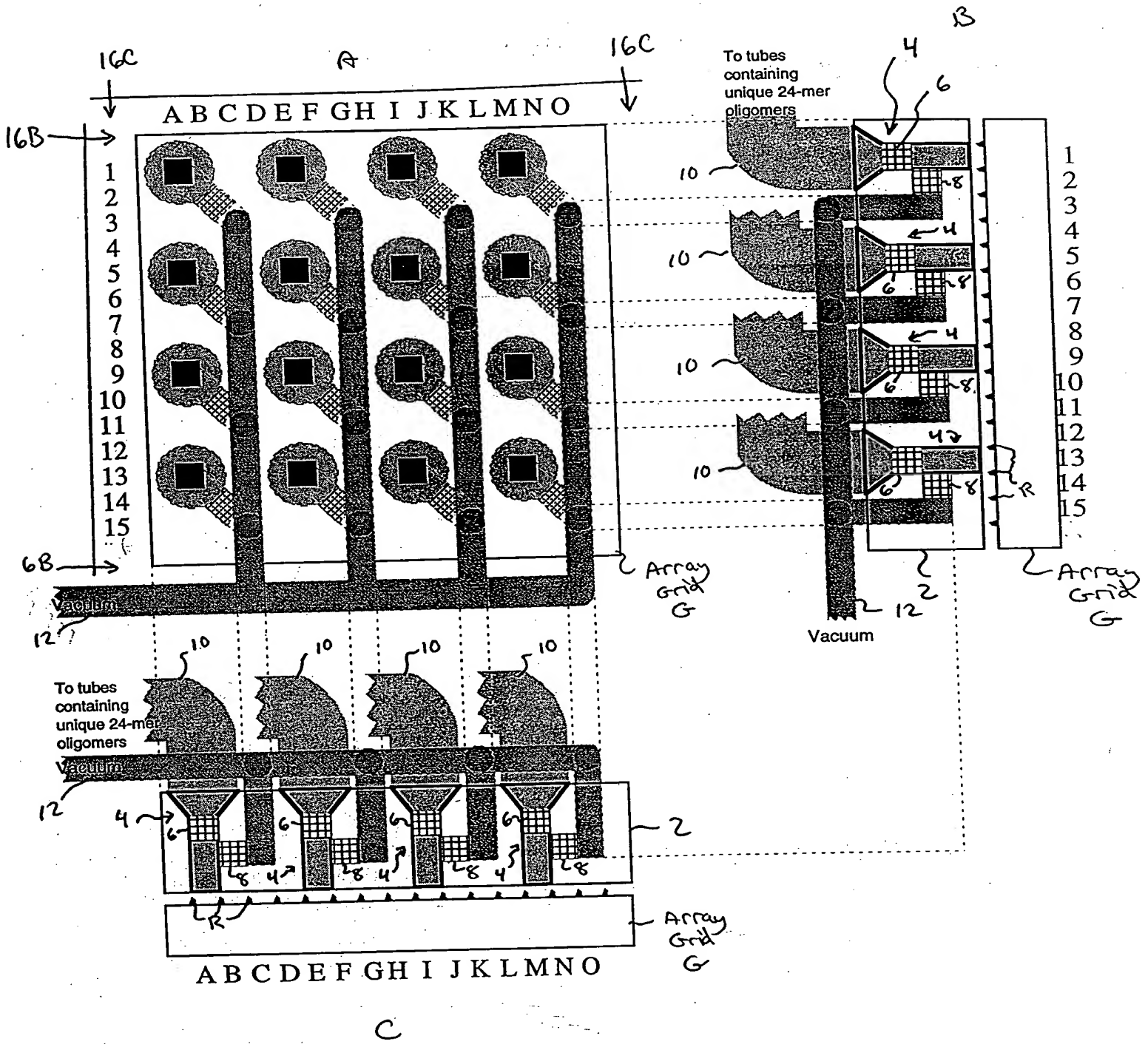

A
B
C
16C
16C
16B
6B
A B C D E F G H I J K L M N O
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Vacuum
12
Array Grid G
To tubes containing unique 24-mer oligomers
10
4
6
8
2
R

FIGURE 16

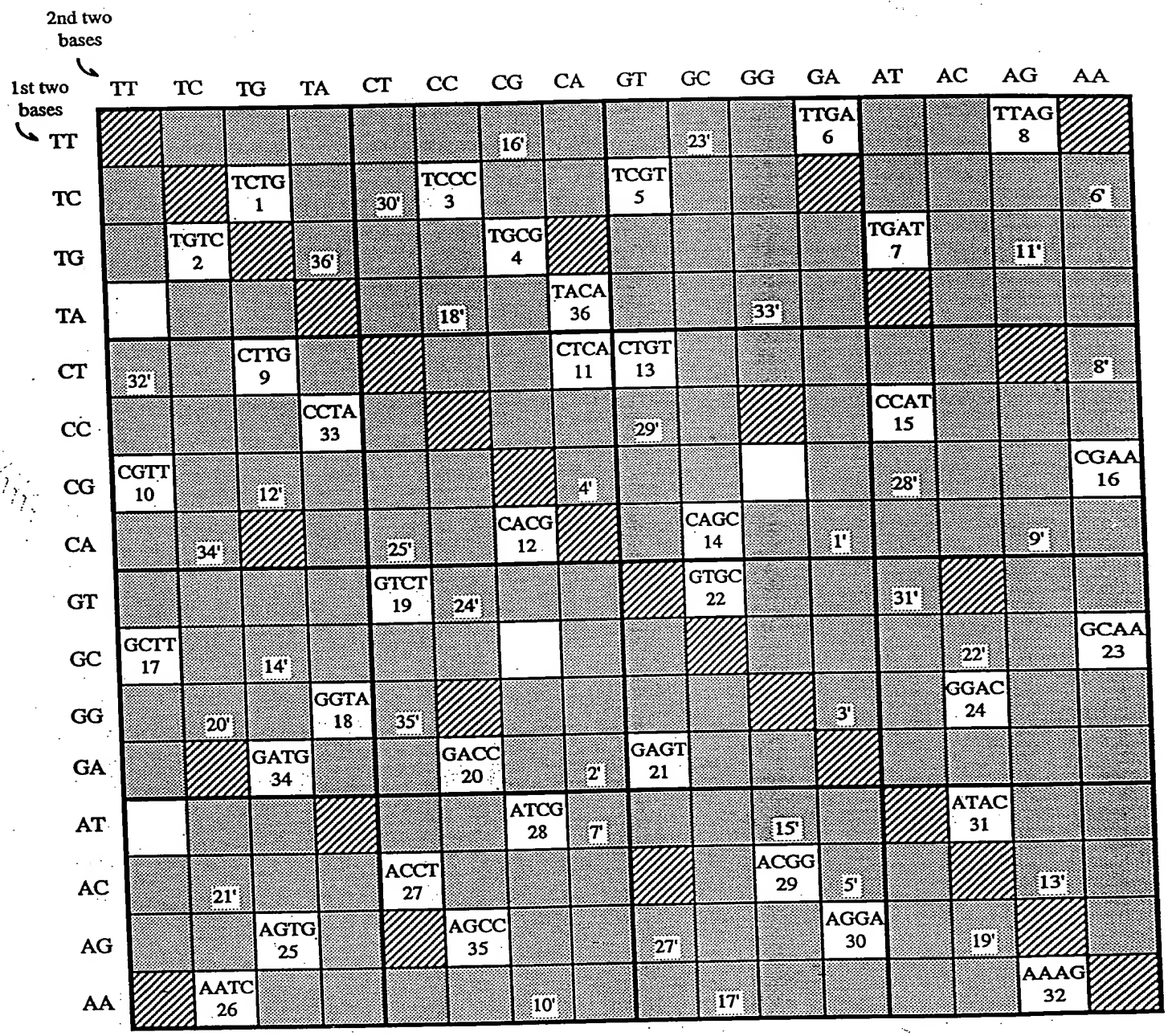

2nd two bases
1st two bases
TT TC TG TA CT CC CG CA GT GC GG GA AT AC AG AA
TT
TC
TG
TA
CT
CC
CG
CA
GT
GC
GG
GA
AT
AC
AG
AA
TTGA 6
TTAG 8
16'
23'
TCTG 1
TCCC 3
TCGT 5
6'
TGTC 2
TGCG 4
TGAT 7
11'
36'
TACA 36
18'
33'
CTTG 9
CTCA 11
CTGT 13
8'
32'
CCTA 33
CCAT 15
29'
CGTT 10
CGAA 16
12'
4'
28'
CACG 12
CAGC 14
1'
9'
34'
25'
GTCT 19
GTGC 22
24'
31'
GCTT 17
GCAA 23
14'
22'
GGTA 18
GGAC 24
20'
35'
3'
GATG 34
GACC 20
GAGT 21
2'
ATCG 28
7'
ATAC 31
15'
ACCT 27
ACGG 29
5'
13'
21'
AGTG 25
AGCC 35
AGGA 30
27'
19'
AATC 26
10'
17'
AAAG 32

FIGURE 17

A

1st Tetramer addition
(columns)

1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5

B

2nd Tetramer addition
(rows)

6	6	6	6	6
5	5	5	5	5
4	4	4	4	4
3	3	3	3	3
2	2	2	2	2

C

3rd Tetramer addition
(columns)

3	4	5	6	1
3	4	5	6	1
3	4	5	6	1
3	4	5	6	1
3	4	5	6	1

D

4th Tetramer addition
(rows)

2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5
4	4	4	4	4

E

5th Tetramer addition
(columns)

6	1	2	3	4
6	1	2	3	4
6	1	2	3	4
6	1	2	3	4
6	1	2	3	4

F

6th Tetramer addition
(rows)

3	3	3	3	3
2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5

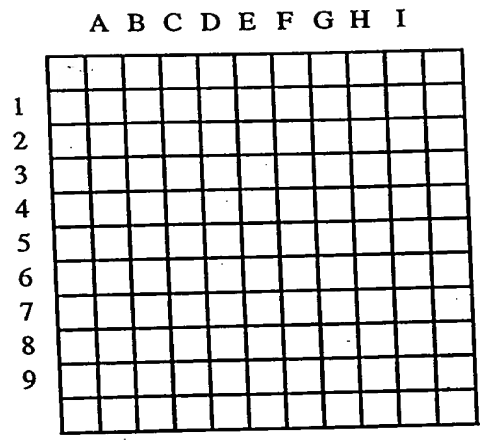
G

Addressable array with full length PNA 24mers

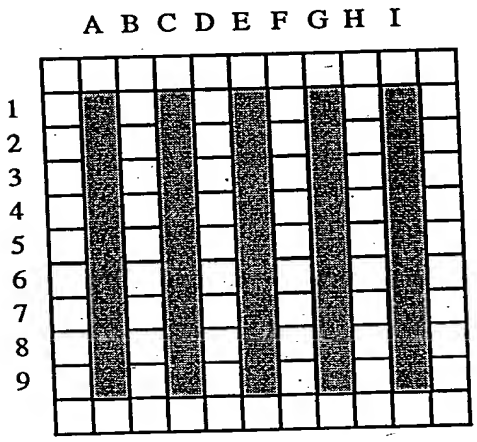
1-6-3-2-6-3	2-6-4-2-1-3	3-6-5-2-2-3	4-6-6-2-3-3	5-6-1-2-4-3
1-5-3-1-6-2	2-5-4-1-1-2	3-5-5-1-2-2	4-5-6-1-3-2	5-5-1-1-4-2
1-4-3-6-6-1	2-4-4-6-1-1	3-4-5-6-2-1	4-4-6-6-3-1	5-4-1-6-4-1
1-3-3-5-6-6	2-3-4-5-1-6	3-3-5-5-2-6	4-3-6-5-3-6	5-3-1-5-4-6
1-2-3-4-6-5	2-2-4-4-1-5	3-2-5-4-2-5	4-2-6-4-3-5	5-2-1-4-4-5

FIGURE 18

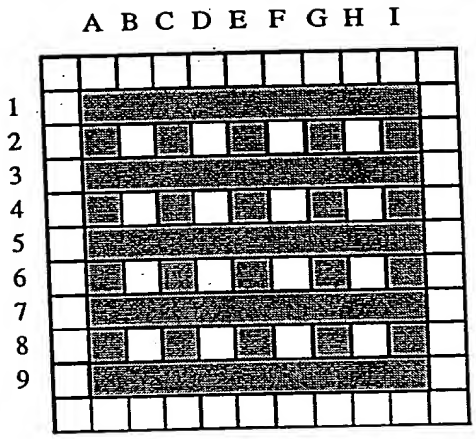
A



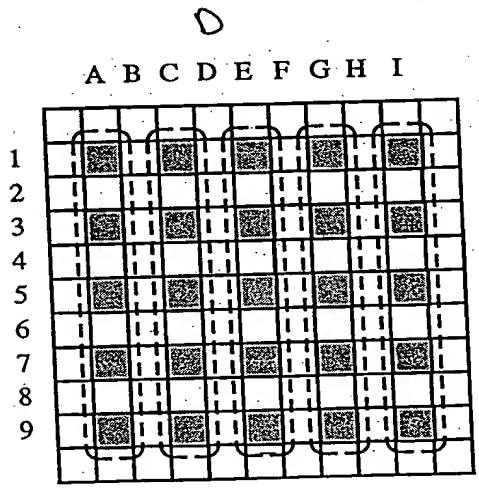
B



C



D



E

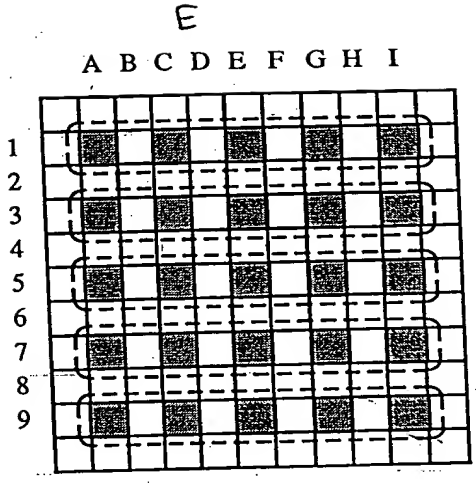


FIGURE 19

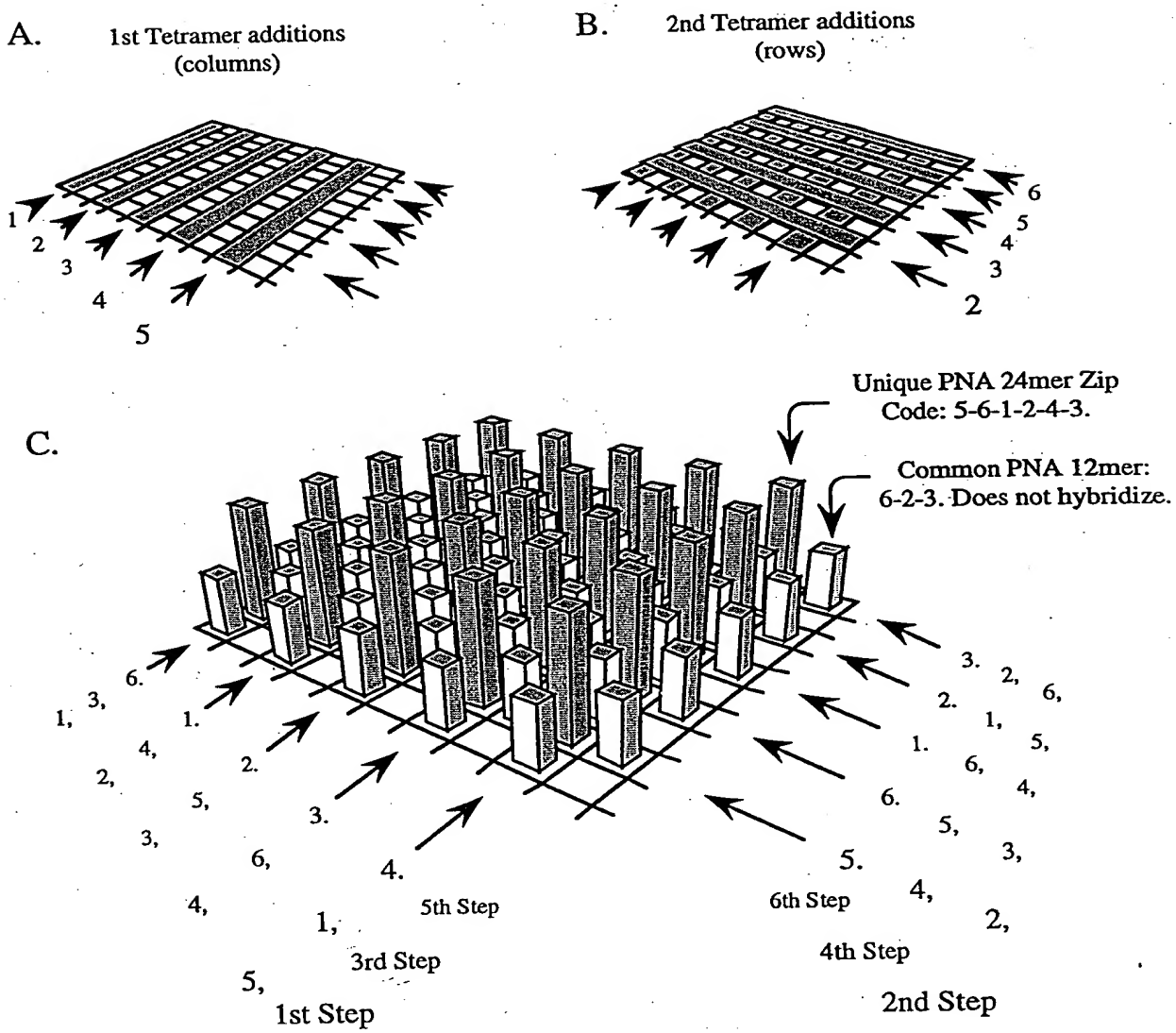

A.
1st Tetramer additions
(columns)
1
2
3
4
5
B.
2nd Tetramer additions
(rows)
6
5
4
3
2
C.
Unique PNA 24mer Zip
Code: 5-6-1-2-4-3.
Common PNA 12mer:
6-2-3. Does not hybridize.
1st Step
2nd Step
3rd Step
4th Step
5th Step
6th Step

FIGURE 20

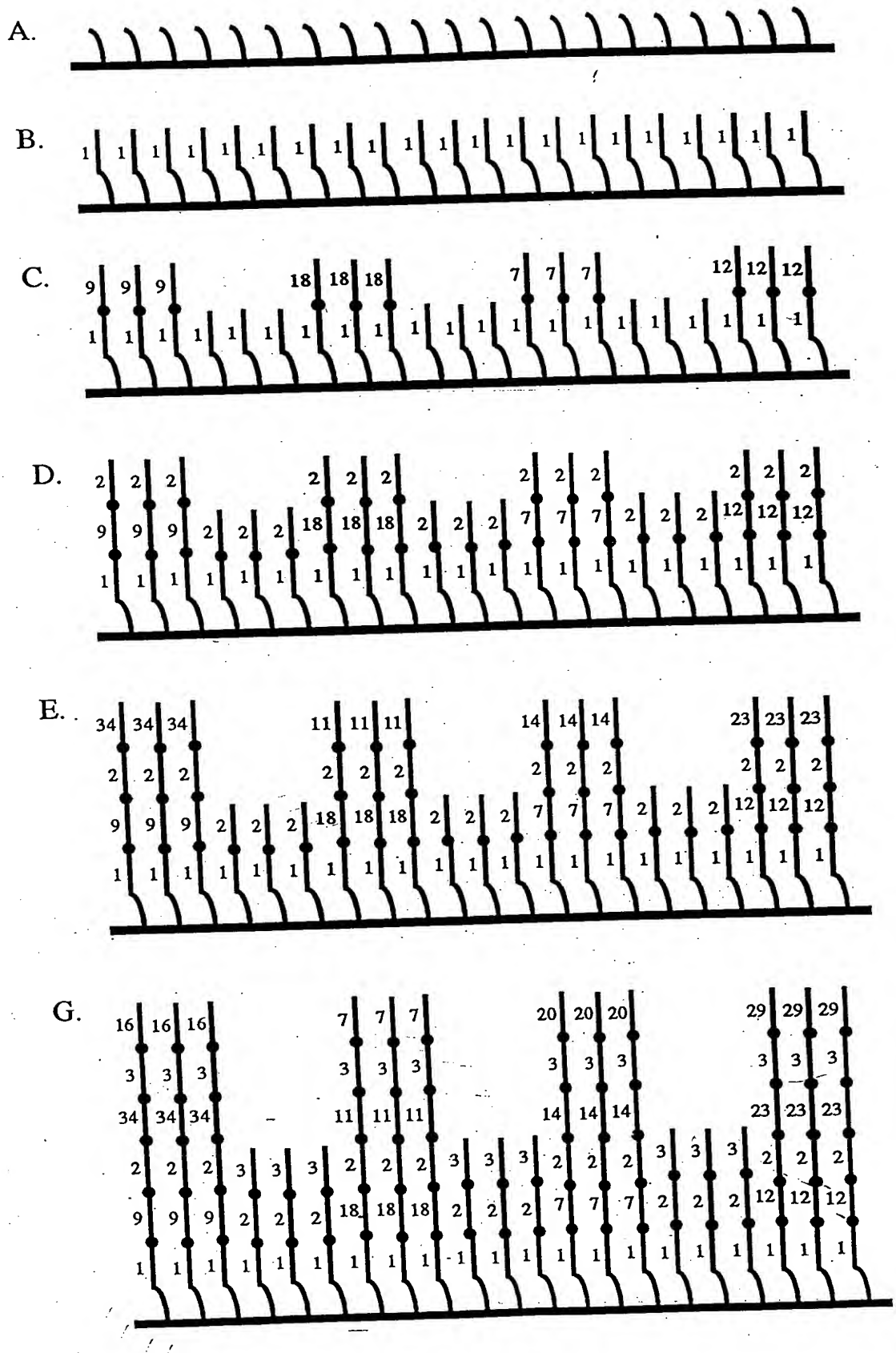


FIGURE 21

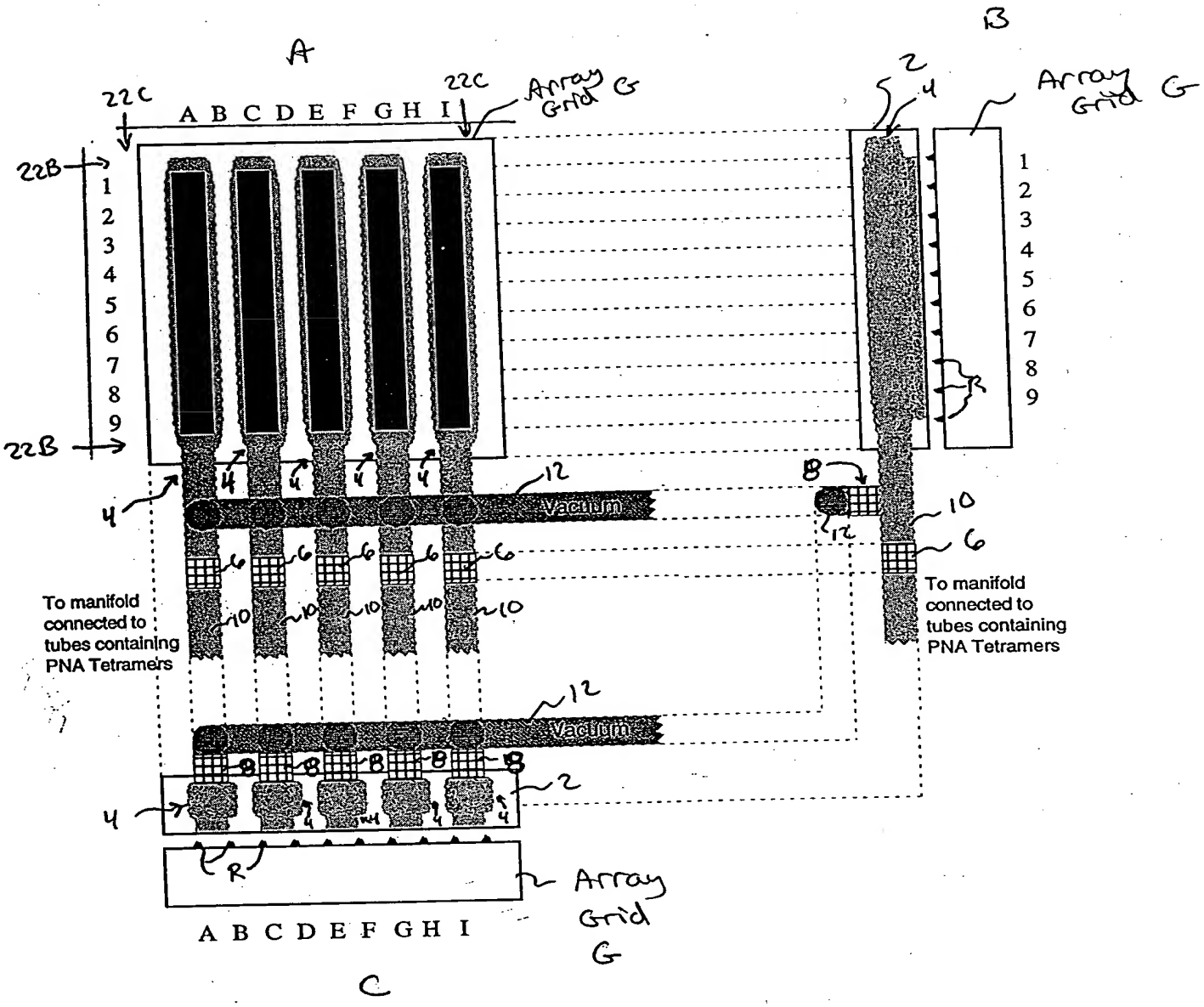

A
B
C
22C
A B C D E F G H I
Array Grid G
22B
1 2 3 4 5 6 7 8 9
Vacuum
To manifold connected to tubes containing PNA Tetramers
To manifold connected to tubes containing PNA Tetramers
Array Grid G
A B C D E F G H I

FIGURE 22

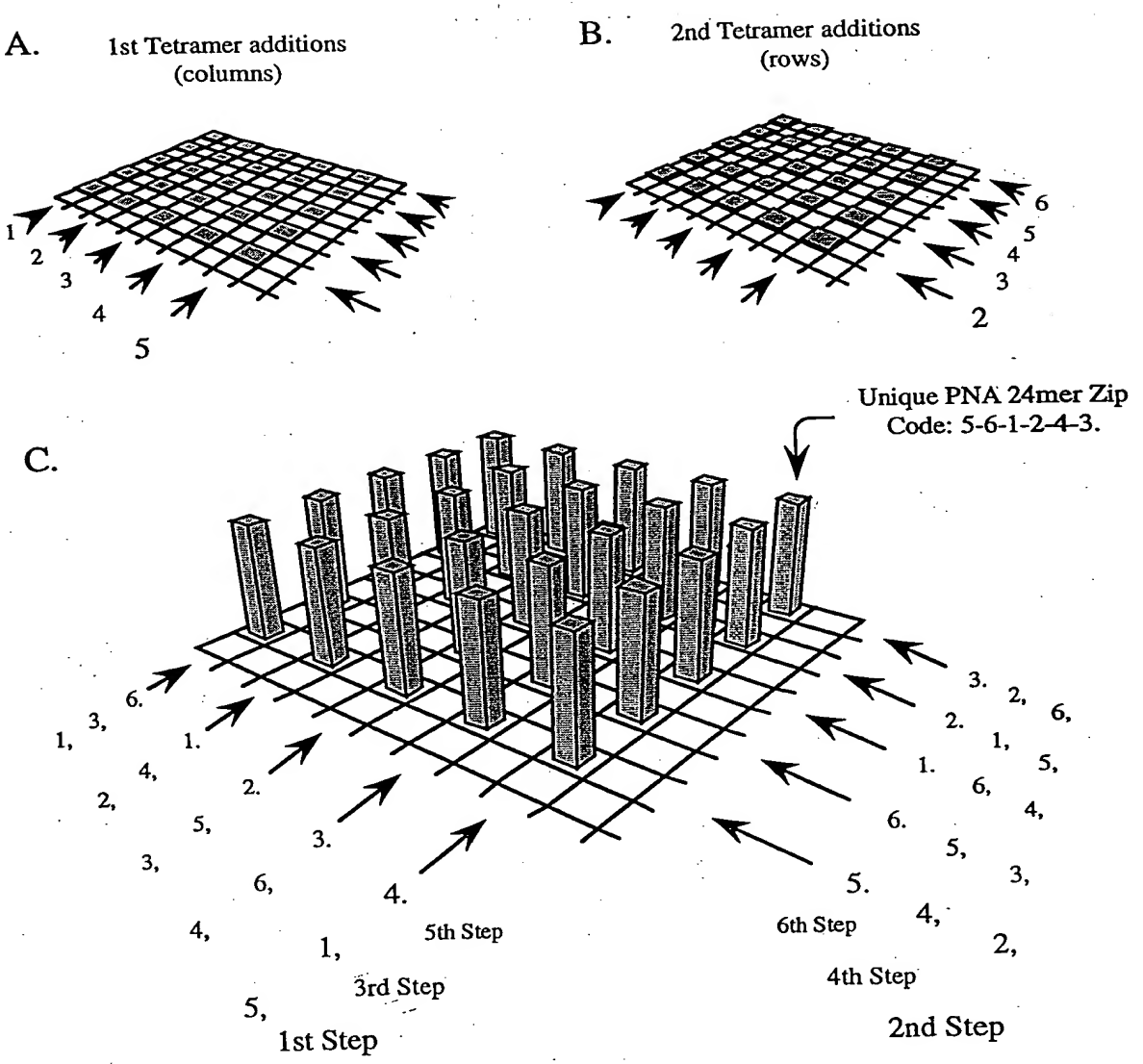

A.
1st Tetramer additions
(columns)
1
2
3
4
5
B.
2nd Tetramer additions
(rows)
6
5
4
3
2
C.
Unique PNA 24mer Zip
Code: 5-6-1-2-4-3.
1st Step
2nd Step
3rd Step
4th Step
5th Step
6th Step

FIGURE 23

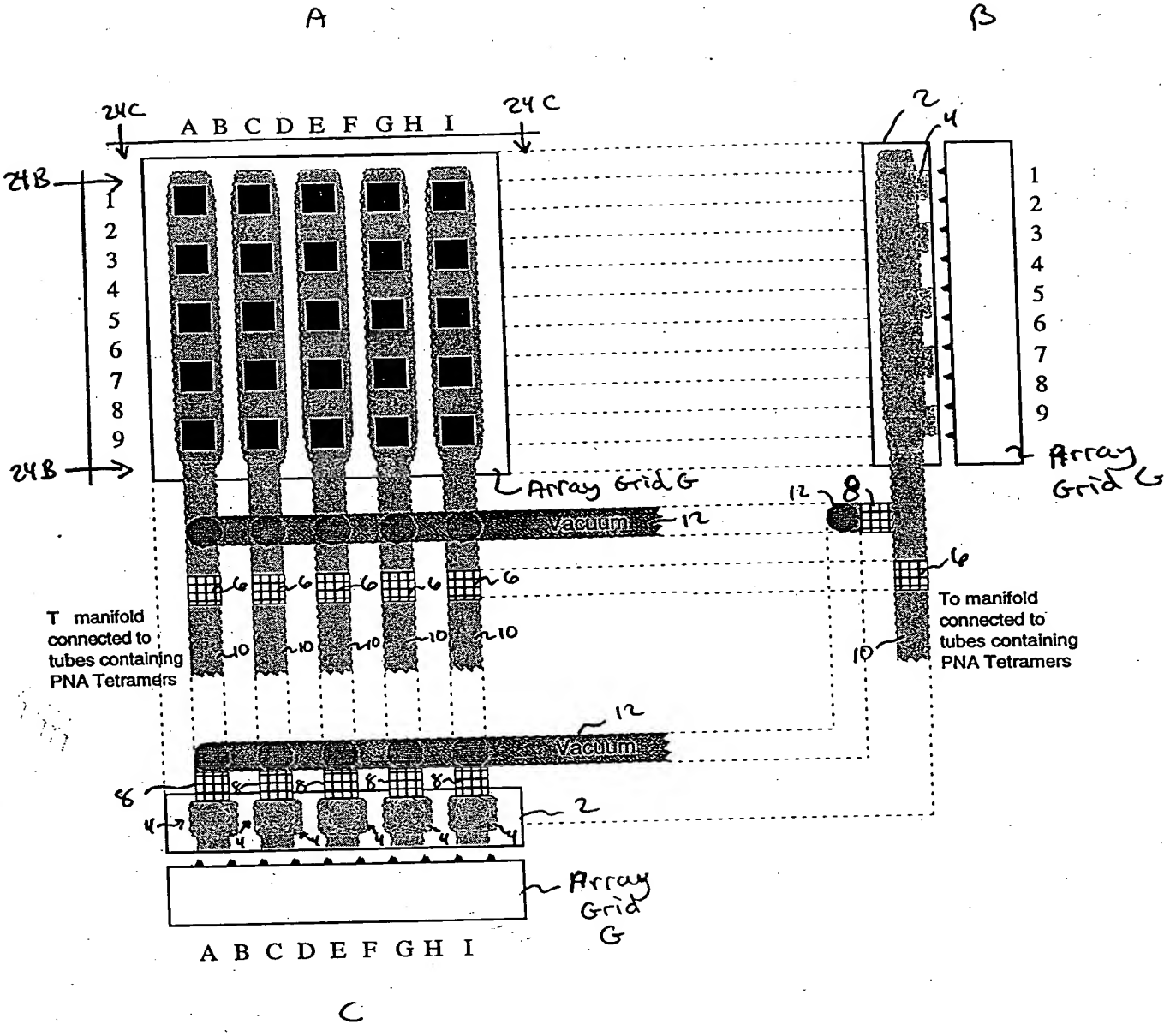
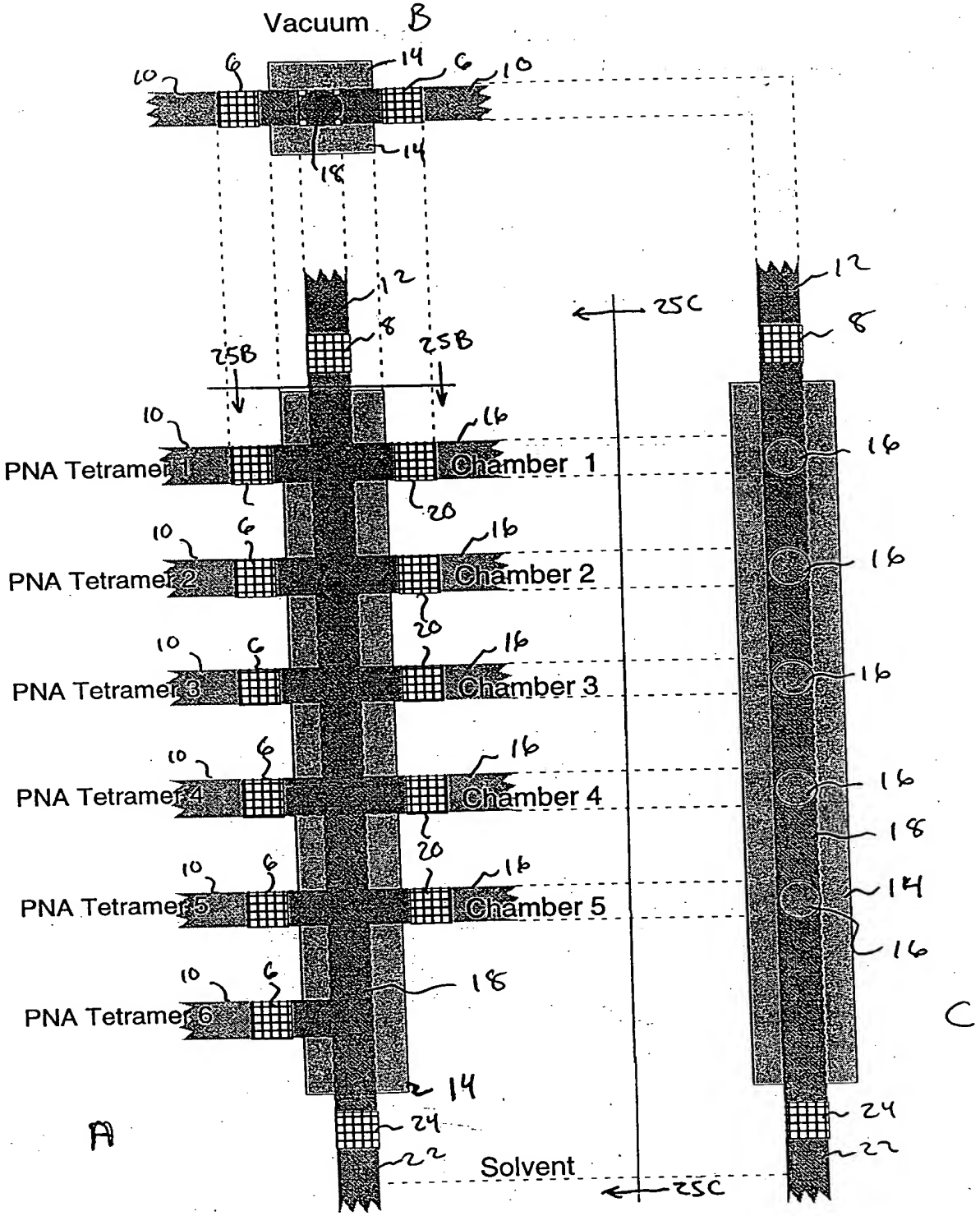

A
B
24C
A B C D E F G H I
24C
24B
1
2
3
4
5
6
7
8
9
24B
Array Grid G
Vacuum
12
8
6
10
T manifold connected to tubes containing PNA Tetramers
To manifold connected to tubes containing PNA Tetramers
Array Grid G
2
4
Array Grid G
A B C D E F G H I
C

FIGURE 24



Valve Block Assembly

6 Inputs & 5 Outputs

FIGURE 25

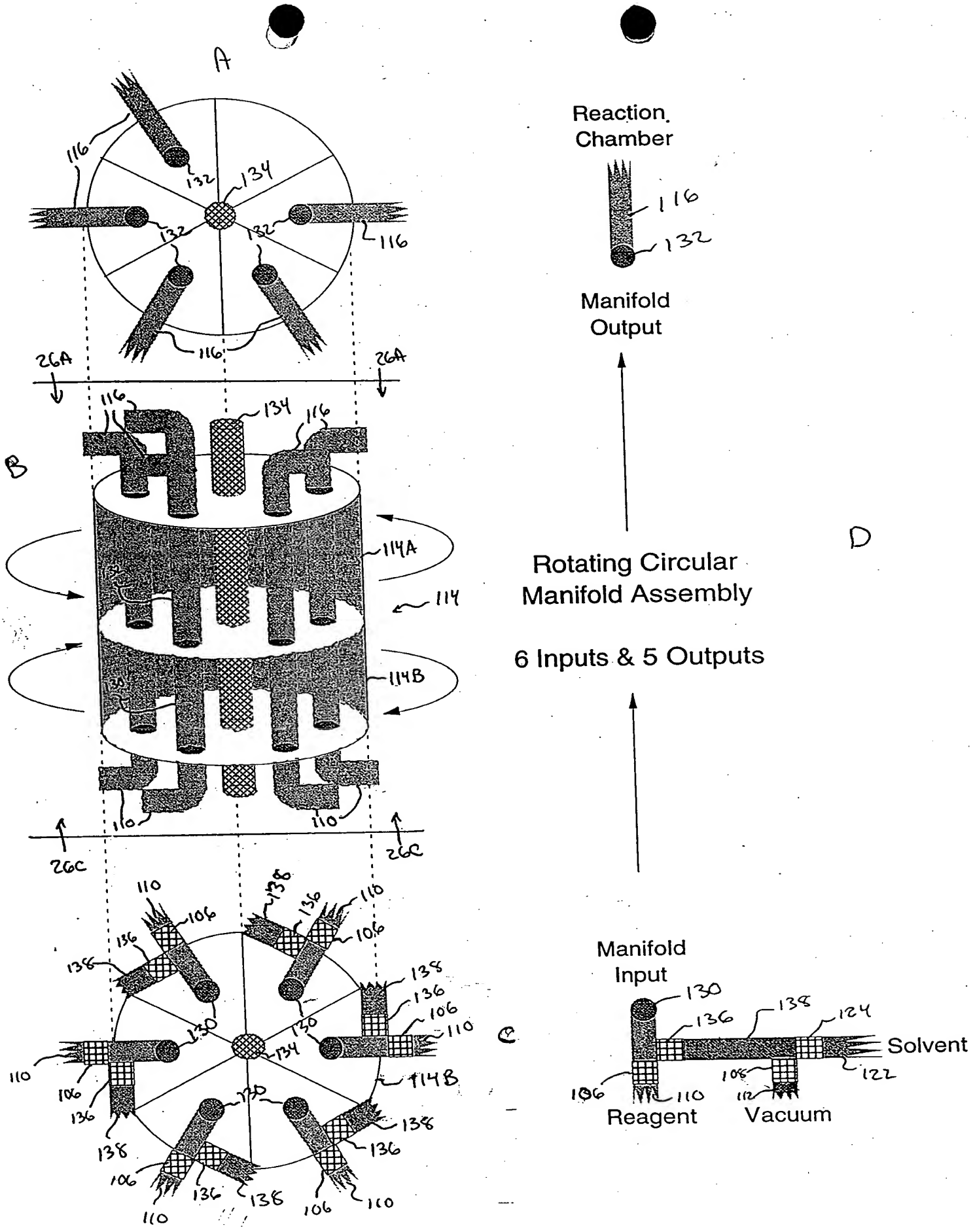

A
B
C
D
Reaction Chamber
116
132
Manifold Output
Rotating Circular Manifold Assembly
6 Inputs & 5 Outputs
Manifold Input
130
136
138
124
Solvent
122
106
110
Reagent
Vacuum
26A
26C
114
114A
114B
134

FIGURE 26

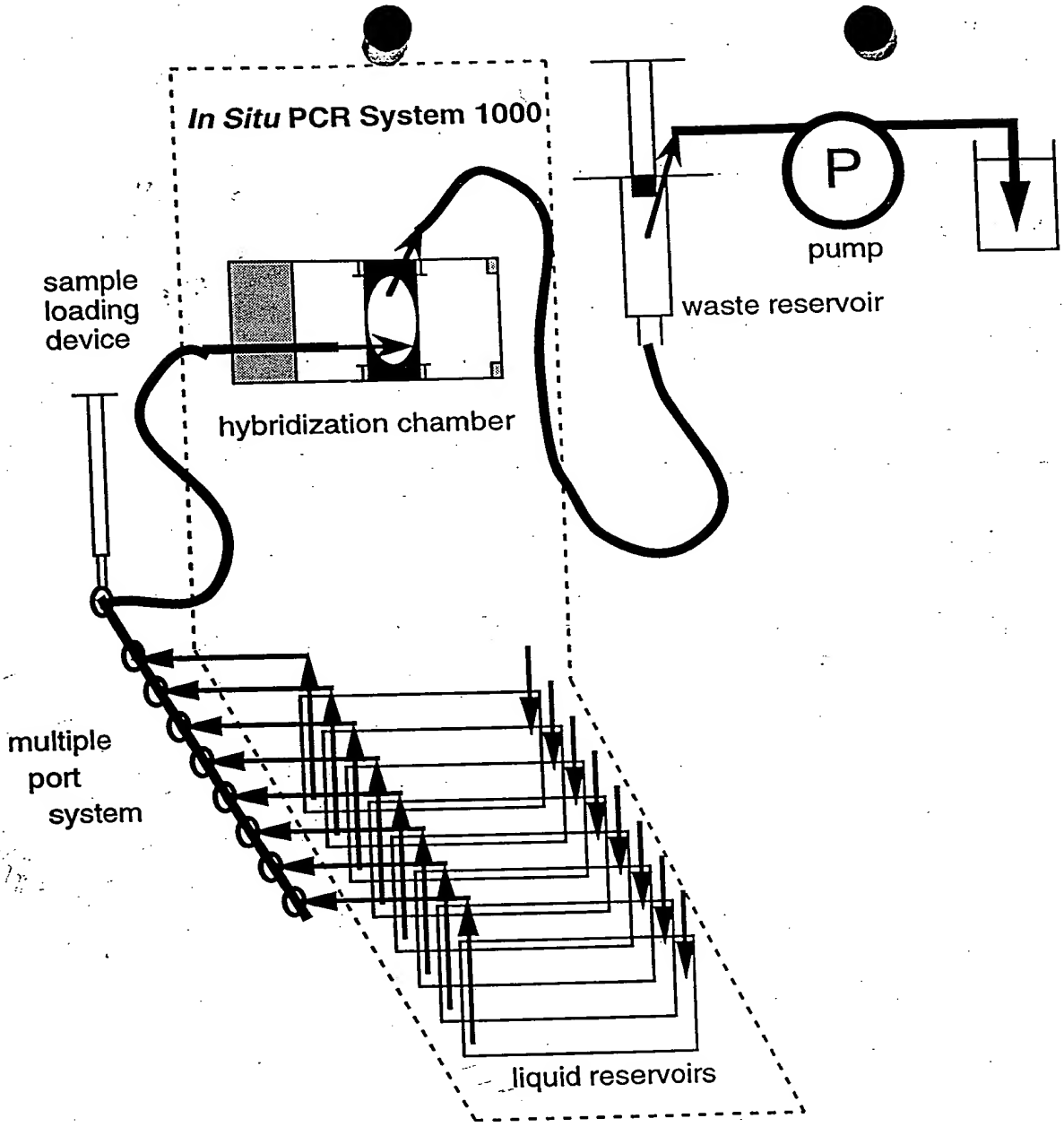

In Situ PCR System 1000
P
pump
waste reservoir
sample
loading
device
hybridization chamber
multiple
port
system
liquid reservoirs

FIGURE 27

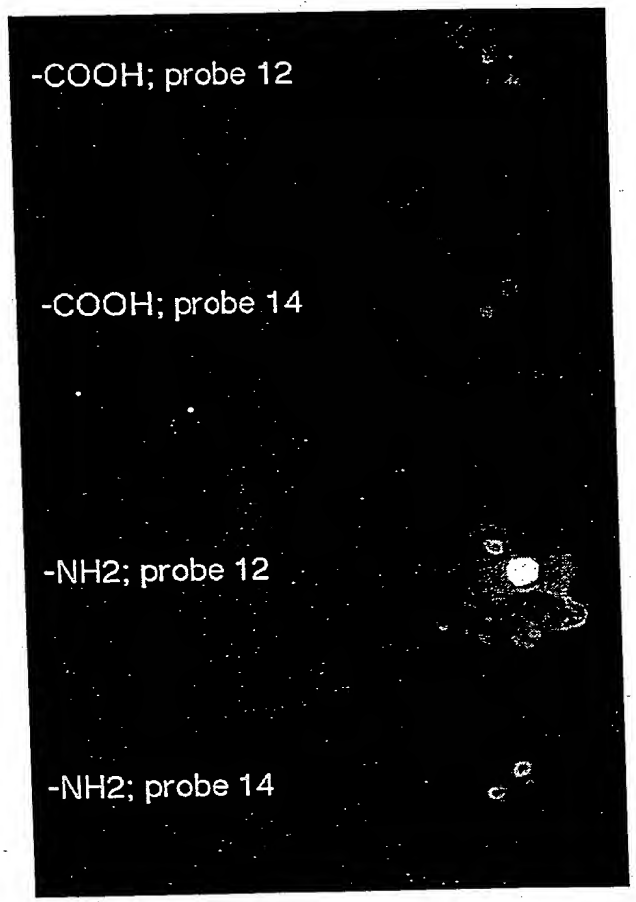


FIGURE 28

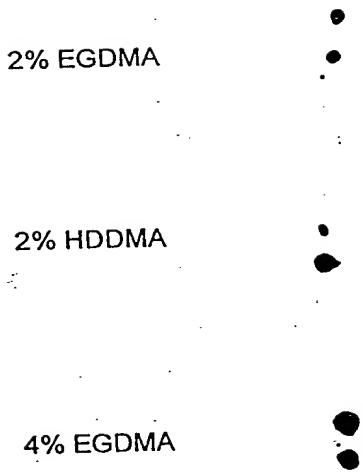


FIGURE 29

1
2

FIGURE 30

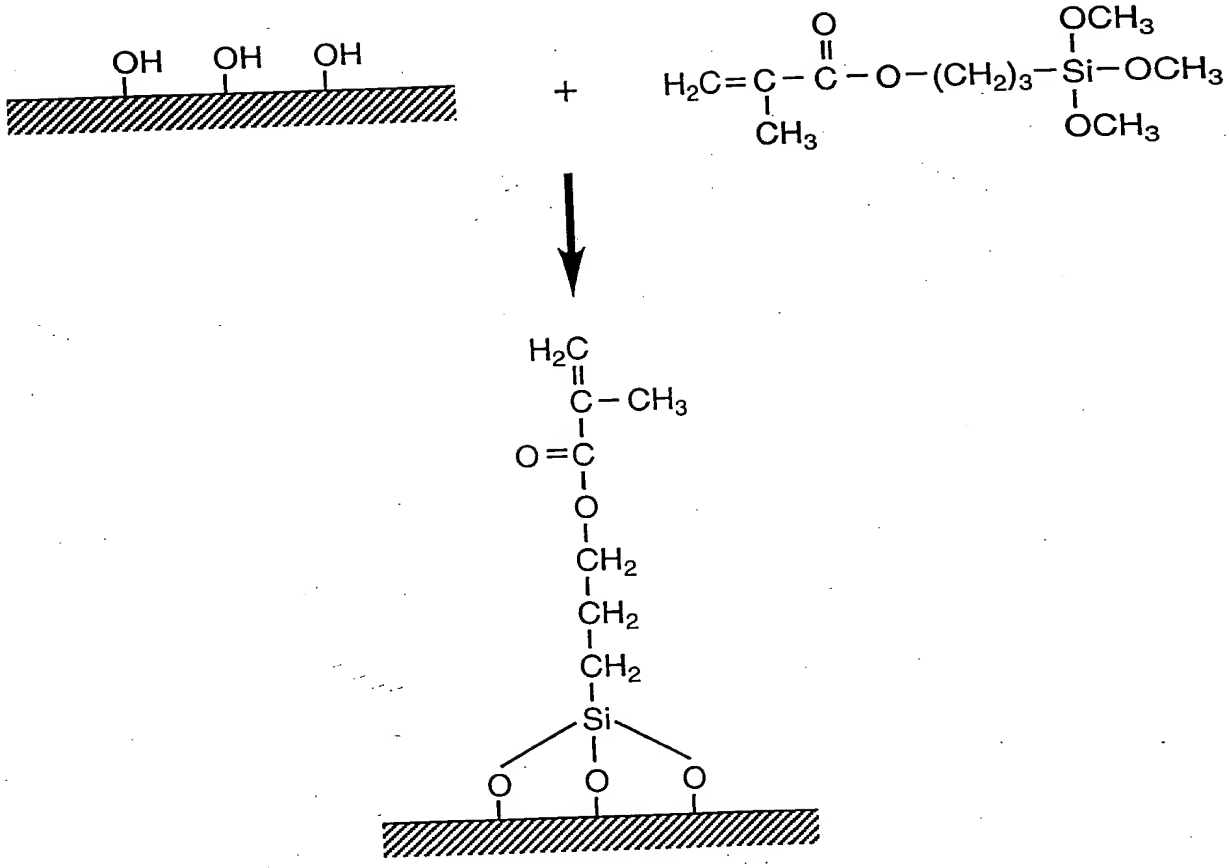

OH
OH
OH
+
O
OCH3
H2C=C-C-O-(CH2)3-Si-OCH3
CH3
OCH3
H2C
C-CH3
O=C
O
CH2
CH2
CH2
Si
O
O
O

FIGURE 31

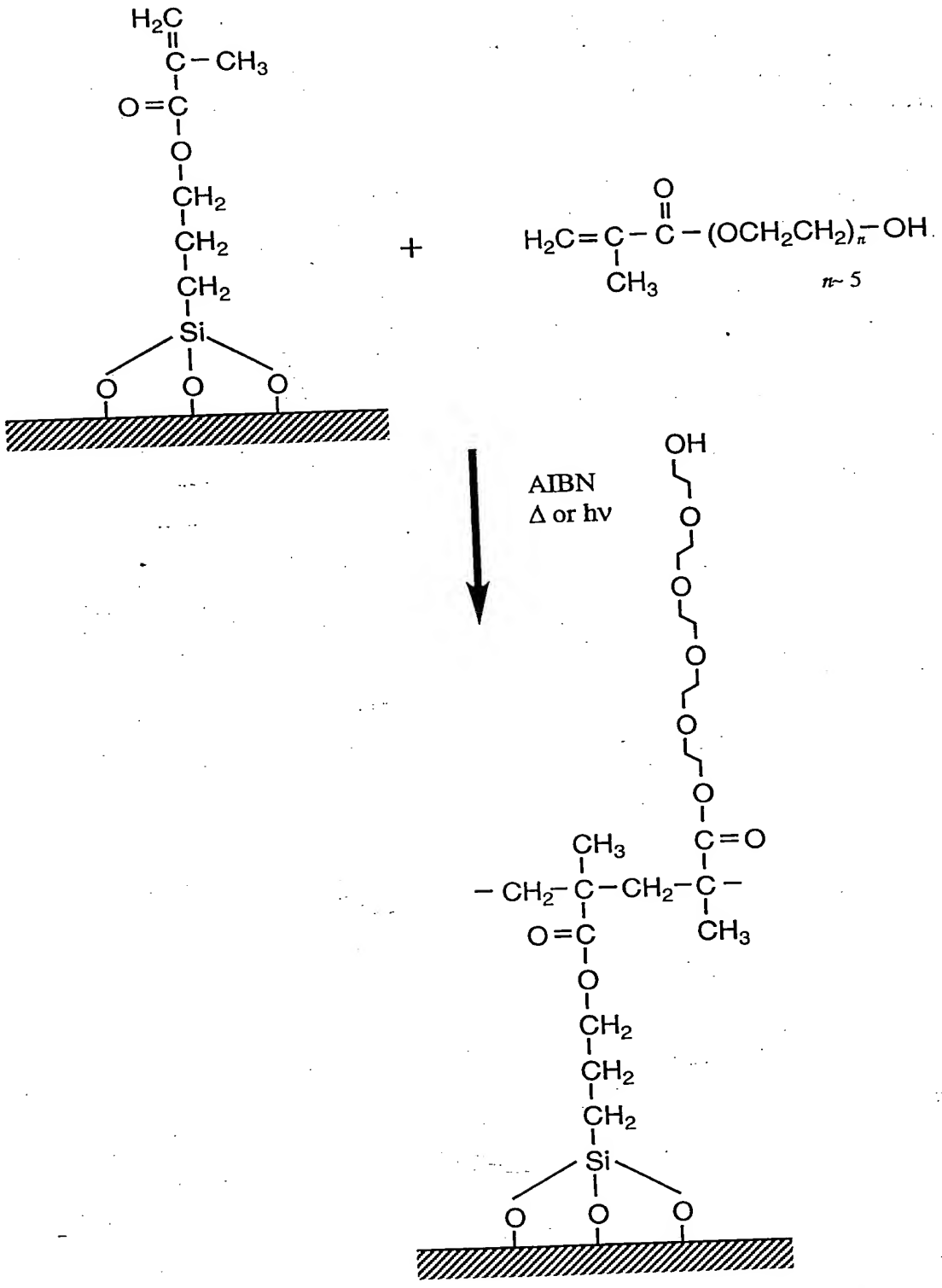


FIGURE 32

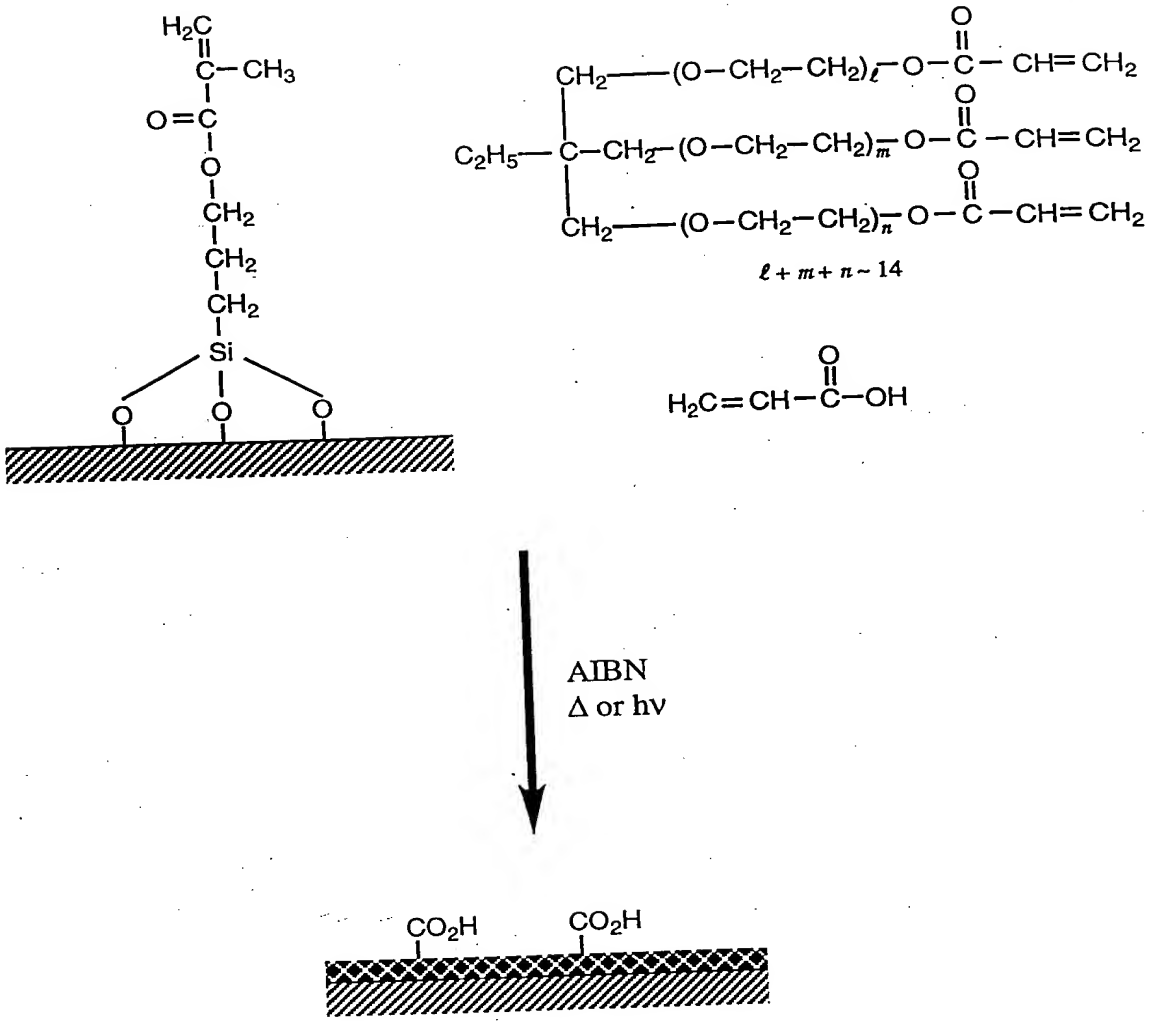

H_2C
C
CH_3
O=C
O
CH_2
CH_2
CH_2
Si
O
O
O
CH_2—(O—CH_2—CH_2)$_\ell$—O—C—CH=CH_2
C_2H_5—C—CH_2—(O—CH_2—CH_2)$_m$—O—C—CH=CH_2
CH_2—(O—CH_2—CH_2)$_n$—O—C—CH=CH_2
$\ell + m + n \sim 14$
H_2C=CH—C—OH
AIBN
Δ or hν
CO_2H
CO_2H

FIGURE 33

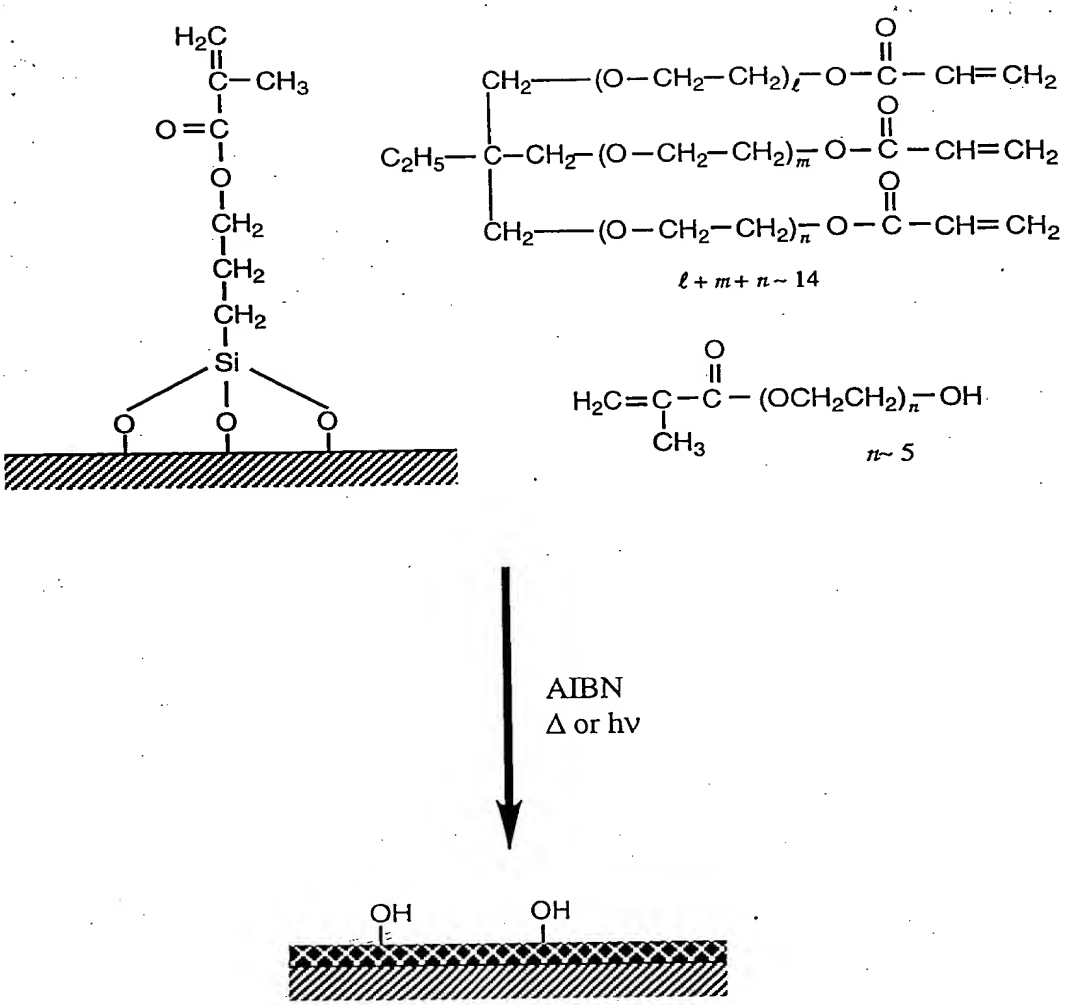

H2C
C–CH3
O=C
O
CH2
CH2
CH2
Si
O
O
O
CH2—(O–CH2–CH2)ℓ–O–C–CH=CH2
C2H5–C–CH2–(O–CH2–CH2)m–O–C–CH=CH2
CH2—(O–CH2–CH2)n–O–C–CH=CH2
ℓ + m + n ~ 14
H2C=C–C–(OCH2CH2)n–OH
CH3
n ~ 5
AIBN
Δ or hν
OH
OH

FIGURE 34